Biological Permeable Reactive Barriers coupled with EK soil flushing for the treatment of diesel-polluted clay soil

Esperanza Mena, Clara Ruiz, José Villaseñor, Manuel A. Rodrigo* and Pablo Cañizares

Chemical Engineering Department. Faculty of Chemical Sciences and Technologies & Research Institute for Chemical and Environmental Technology (ITQUIMA). Universidad de Castilla La Mancha, Campus Universitario s/n.13071, Ciudad Real, Spain.

*Corresponding author: Tel.: +34 926 29 53 00 Ext. 3411; Fax: +34 926 29 52 56. E-mail address: Manuel.Rodrigo@uclm.es

Abstract

Removal of diesel from spiked kaolin has been studied in the laboratory using an innovative technology coupling electrokinetic soil flushing (EKSF) and biological permeable reactive barriers (Bio-PRBs). Development of the Bio-PRB was done using a culture of active sludge from an urban WWTP and clean kaolin soil as support. Bio-PRB was placed in the middle point of the soil section submitted to an electric field (1 $V \cdot cm^{-1}$). This is the farthest point to the electrodic wells and the influence of the strong pH variations that can make unviable the biological degradation process is minimum. Using this configuration, after two weeks of operation, 30% of diesel is removed with an energy consumption under 70 $kWh \cdot m^{-3}$.

Highlights

Pollutant biological degradation process in a Bio-PRB can be improved using EKSF.

The location of the Bio-PRB is the most important key for the best performance of the microorganisms.

With an energy consumption under 70 kWh·m^{-3}, 30% of the diesel pollution is removed from the clay soil.

Keywords

Permeable reactive barriers, electrokinetic soil flushing, bioremediation, biobarriers

1. Introduction

Fuel pollution in soil is usually related to accidental leaks during handling, transport, or storage activities in underground storage tanks, distribution systems, or crude oil refining industrial activities. This is a serious environmental issue because of the high negative impact of fuel pollution on the quality of water reservoirs, which prevents the use of this water for human consumption, and the hazardousness of the chemical species contained in the fuel for organisms in the soil. Accordingly, many technologies have recently been assessed to remove this pollution efficiently, [1-5] including bioremediation and electrokinetic soil flushing (ESKF). Bioremediation is a very effective group of technologies based upon the degradation of pollutants by microorganisms. Because the efficiency of microorganisms depends on many factors (temperature, pH, nutrients, electron acceptors, etc.), maintaining optimal conditions for microbial degradation is not an easy task, particularly in soils with low permeability, in which transport of species is very limited [6-8]. EKSF consists of the use of a flushing fluid to drag pollutants from the soil combining efficiently the different electrokinetic mass transport processes (electro-osmosis, electromigration and electrophoresis) and also taking advantage of other processes, such as water electrolysis and ohmic heating which develops when an electric field is applied to a soil [9-11].

Coupling EKSF and bioremediation could result in a promising technology because the enhanced mass transport obtained with EKSF could be greatly helpful for effective pollutant degradation carried out during bioremediation, leading to a more effective technology compared with both separately treatments [12-15]. The principal advantage of coupling the biological treatment with the EKSF is that pollutants are degraded in situ by the microorganisms, and an ulterior treatment of the washing solutions is not necessary. However, when the single EKSF treatment is applied for the transport of pollutants, they are accumulated in the anolyte or in the catholyte, obtaining high volumes of dissolutions highly polluted. However, to promote synergy between both technologies, conditions should be carefully assessed due to the huge differences between conditions required for bioremediation technologies (mild conditions with good distribution of nutrients) and those obtained from EKSF (harsh conditions with large pH and temperature gradients), specially applying large electric fields, which could result in an antagonistic combination if not enough attention is paid to operation conditions [16].

Basically, the advantages looked for in electrokinetic enhanced bioremediation are based on increasing the biological pollutant removal rate via the electrokinetic transport phenomena [17, 18]. In previous works, the electro-migration process has been used for supplying inorganic nutrients to soil bioremediation processes [6, 8, 19, 20]. The electrophoresis process has been suggested for the transport of microorganisms to increase the rate of the biological degradation process [16]. Electro-osmotic drag has been applied for the drag of non-charged water soluble species (pollutants or even microorganisms) [14, 21-24]. Heating (caused by the high ohmic drops when an electric field is applied to a soil) has been used to increase the rate of bioremediation processes in cold climate areas [25]. However, not all results were positive because it is also well known that the application of an electric current in soil, apart from the mobility and the

heating processes, also results in a number of negative consequences for biological processes. Specifically, water electrolysis reactions (Equation 1 and 2) that occur on the surface of the active electrodes result in the formation of an extremely acidic and basic pH in the areas near the anode or cathode, respectively. In these areas, viability of the microbial consortia used in this work, which it is not acclimated to degrade the pollutant in extreme pH conditions, is not possible and, consequently, biological degradation process will be inhibited.

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \quad (1)$$

$$H_2O - 2e^- \rightarrow \tfrac{1}{2}O_2 + 2H^+ \quad (2)$$

Furthermore, electrokinetic mobility phenomena have been previously suggested to have a positive effect on the biological degradation process due to the increase in the possibilities of interaction between the different elements that take part in the microbial metabolising process [26-29]. However, in many cases this is an idealistic situation, and the electrokinetic transport rates at which these elements move through the soil matrix are very different [15]. This can result in removal from the soil, decreasing the possibilities of interaction between them. In this case, contrary to what is expected, the rate of the biological degradation process will be undesirably inhibited.

In this work, the coupling of the EKSF technology with a biological degradation system through the use of Bio-PRBs, or biobarriers, is suggested. Thus, to avoid the negative effects of the electrokinetic technology, the pollutant-degrading microbial consortium was placed far away from the areas of more extreme negative conditions for the degradation process (those near to the electrodes). To do this, at the beginning of the experiment, a permeable reactive barrier (PRB) with activated sludge mixed with the soil

was placed in an intermediate section of the polluted area. The microbial consortium is not attached on a solid support but it is spread on a portion of soil, so the access to other positions of the polluted soils is easier. The goal of this manuscript is to assess the potential of combining EKSF with bioremediation using novel Bio-PRBs. This experiment was carried out in a bench scale setup. Electrolyte wells were monitored daily, while soil will be fully characterised using post-study characterisation.

2. Experimental

Lab-scale set-up is schematised in Figure 1. It was made of transparent methacrylate and divided into seven compartments. The Bio-PRB was loaded in the central compartment. On the compartments situated on both sides of the biobarrier, and separated by nylon mesh (0.5 mm mesh size), the diesel-polluted soil was manually loaded and compacted. The electrode compartments were on one side of each section of polluted soil. They were also separated from the soil by a 0.5 mm nylon mesh. One of these compartments served as anode and the other as cathode. Each electrode compartment had an overflow to collect the water transported because the electroosmosis process.

Graphite electrodes were used and connected to the power supply (HQ Power, Gavere, Belgium) so that one of them constituted the anode and the other one the cathode. Dimensions of these electrodes, provided by Carbosystem (Madrid, Spain), were 10.0 × 10.0 × 1.0 cm^3. In this way, they had the same cross section as the fraction of soil to be treated, which maintained a homogeneous distribution of current lines throughout the soil. Kaolinite, provided by Manuel Riesgo Chemical Products (Madrid, Spain), was used as a model of clay soil. Properties of this synthetic clay soil were provided by the commercial supplier, and are detailed in Table 1. The procedure used to pollute the soil consisted on diluting tenfold the diesel in acetone and evenly, drop by drop, distributing

this solution in the corresponding amount of kaolinite. The solvent was allowed to evaporate at room temperature for at least one day. The concentration of diesel present in the soil at the beginning of the experiments was fixed by the authors at 10 $g \cdot kg^{-1}$. This value is similar to other usually find in bibliography.

The solution used as anolyte and to moisten the soil and ensure the conductivity of the same has the following composition, which is very similar to tap water but without disinfecting chlorine species: 30.36 $mg \cdot L^{-1}$ of $NaNO_3$ (which corresponds with 5 $mg \cdot L^{-1}$ of N), 70 $mg \cdot L^{-1}$ of $NaHCO_3$ (10 $mg \cdot L^{-1}$ of C), and 88.75 mgL^{-1} of Na_2SO_4 (20 $mg \cdot L^{-1}$ of S). The polluted clay soil was wetted up to saturation conditions, with a moisture content of approximately 40% prior to be compacted in the set-up. In the cathodic compartment an anionic surfactant solution was used as catholyte. A 2.38 $g \cdot L^{-1}$ sodium dodecyl sulphate (SDS), provided by Panreac Chemical Products (Barcelona, Spain) solution was loaded in the cathodic compartment at the beginning of the experiment. This solution was used as the flushing liquid to slowly dissolve diesel in the water containing in the soil pores as the surfactant moved through the soil. Thus, displacement of the organic substrate to the Bio-PRB treatment area was accelerated and degradation of the same was carried out.

The procedure for the development of the biobarrier consists of mixing an amount of the same non-polluted kaolinite with the corresponding volume of active sludge to achieve the same moisture in the Bio-PRB as in the rest of the polluted soil (approximately 40%). This active sludge was obtained from the biological reactor of the WWTP of Ciudad Real, Spain. The total volatile solids concentration of the active sludge was approximately 10 $g \cdot L^{-1}$ and the total volatile solids concentration in the Bio-PRB at the beginning of the treatment was 4.5 $g \cdot kg_{Soil}^{-1}$. Previously to be mixed with the non-polluted kaolinite soil

that acts as support of the microbial consortium in the biobarrier, active sludge was supplemented with Bushnell-Hass Broth (BHB) nutrient medium (DIFCO™, Le Pont de Claix, France). This procedure was used to ensure that enough inorganic nutrients were available for the microorganisms to perform the degradation of the organic substrate. This medium consisted of a mixture of inorganic nutrients, which composition in $g \cdot L^{-1}$ was: 0.2 $MgSO_4$, 0.02 $CaCl_2$, 1 KH_2PO_4, 1 $(NH_4)_2HPO_4$, 1 KNO_3, and 0.05 $FeCl_3$.

The soil was manually compacted into the compartments on either side of the Bio-PRB, in an attempt to achieve the highest degree of compaction possible to avoid the formation of preferential paths that could interfere with the results. Afterwards, the biobarrier was loaded into the central compartment. Finally, both electrodic compartments were filled with the appropriate electrolyte.

The experiments were performed in a potentiostatic mode, i.e., setting a voltage gradient value, which remained constant throughout the experiment, while the current intensity value varies with time, depending on the characteristics of the medium. In this case, the voltage gradient used was 1 $V \cdot cm^{-1}$. Taking into account the length of the section treated (20 cm) the total value of the fixed voltage was 20.0 V. The duration of the experiment was two weeks.

Daily, the electrical current, the temperature on the biobarrier, the electroosmotic volume removed from the cathodic collector and the pH, conductivity and phosphate, nitrate, ammonium, diesel, and SDS concentrations of the electroosmotic fluid were monitored. The liquids contained in the electrolyte wells were also daily monitored measuring pH, conductivity, phosphate, nitrate, ammonium, diesel, and SDS concentration. On the other hand, the following parameters were measured in the soil and in the Bio-PRB at the beginning and end of the experiment (post-study characterisation): pH, conductivity,

humidity and microorganisms, phosphate, nitrate, ammonium, diesel, and SDS concentrations. At the end of the experiment, both sections of the polluted soil were divided into eight portions so that the measurements of the mentioned parameters were performed at 16 points of the soil, as it is schematized in Figure 1 part b. In this way, the influence of the position of the electrode in the measured parameters and also the axial dispersion in the points situated at the same distance of the electrodes was analysed. Bio-PRB was considered entirely as a unique section.

Following the experimental procedures for the measurement of each parameter are detailed:

- Measurement of the moisture was carried out by drying the soil samples in an oven for 24 hours at 105ºC. Moisture was calculated taking into account the weight difference in the samples before and after drying.
- Nitrates, phosphates, ammonium, and SDS concentrations were measured from dried soil samples. Soil samples (10 g) were suspended in 25 ml of Milli-Q water by 20 min of vigorous magnetic agitation. Afterward, samples were centrifuged at 4000 rpm for 15 min. The supernatant phase was used for the measurements. Inorganic ionic nutrients concentrations were measured using the Gallery photometric analyser (Thermo Fischer Scientific, Massachusetts, USA). SDS concentration was measured using a specific photometric method previously detailed in bibliography [30, 31].
- Diesel concentration was determined using a fractionated serial extraction. Wet soil samples (10 g) were mixed with 6 ml of hexane (divided in three steps of 2 ml). In every extraction step the soil was mixed with the corresponding volume of dissolvent and agitated vigorously in a Vortex

agitator for 5 min. After that, samples were centrifuged at 4000 rpm for 15 min. Samples taken from the organic supernatant phase were analysed using a Trace GC Ultra gas chromatograph equipped with a flame ionisation detector (GC-FID) (Thermo Fischer Scientific, Massachusetts, USA). Analysis were done in triplicate, drawing three identical samples in each sampling point.

– Microorganism concentration was measured by suspending 10 g of wet soil in 10 ml of saline by 1 min of Vortex agitation. Subsequently, a 100 µL aliquot of the soil-saline suspension was plating onto Petri dishes with non-selective solid bacterial growth media. The nutrient soil phase of these dishes was prepared using LB medium (with the following composition per litre of deionised water: NaCl 10 g, yeast extract 5 g and casein peptone 10 g), 15 $g \cdot L^{-1}$ of European Bacteriological Agar and 2 $g \cdot L^{-1}$ of glucose as the carbon source. Inoculums were evenly spread using Digralsky handles and the plates were incubated for 48 hours at 26ºC, which is the necessary time to enumerate the individual colonies present in each sample. Analysis were done in triplicate, drawing three identical samples in each sampling point.

– pH, conductivity and dissolved oxygen concentration were measured using the corresponding selective electrodes.

3. Results and Discussion

The main objective of this work is to improve the biological degradation of diesel pollutant in the soil. Electrokinetic processes are used for increase the transport rate of the pollutant to the Bio-PRB. However, the application of an electric field causes important modifications in the properties of the soil. Following, variations observed in

the system with the treatment are analyzed, paying special attention to the more important variables influencing the performance of the pollutant degradation.

Figure 2 shows the changes in the main operating parameters of the treatment, including electrical current density, temperature at the Bio-PRB, pH and conductivity of the electrolyte contained in the anodic and cathodic wells during the two-week long remediation test.

Regarding the current density, it decreases abruptly from the beginning of the test down to a constant value of approximately 1 $A \cdot m^{-2}$ in 4 days, indicating that the rate of electrolytic and transport processes should change in the same fashion. Temperature in the Bio-PRB is kept approximately constant during the whole treatment, suggesting that increases in temperature due to ohmic losses are balanced with heat losses to the environment.

Regarding pH in the electrolyte wells, as expected it changes abruptly in both the cathodic and anodic wells due to the well-known reduction (Equation 1) and oxidation (Equation 2) of water, respectively. These changes in pH can explain the increase in conductivity observed in the Figure 2 as well, although in this case, transport of other ions to and from the electrodic wells explain the slower stabilisation of the dynamic response (longer settling times) in the steady-state values.

Figure 3 compares the main flow rates and the resulting fluxes measured from the experimental setup during the experiment. Although it is almost negligible, this also includes the volume of samples taken each day to characterise the system because these data have also been considered in the mass balance calculations. As can be observed, the main flows in the cell are the fluid taken at the cathode (related to the electro-osmotic

flux), the fluid added to the anodic well (related to the electro-osmotic and the evaporation fluxes), and the fluid evaporated (calculated by mass balance). The main flux is the electroosmotic flux from the anode to the cathode. As suggested before, its value decreases with the current density of the system during the first five days and then stabilises at a rate of approximately 0.4 $cm \cdot d^{-1}$ (flux has been calculated referring the flow rate to the cross area of the setup). The other important flux to be considered is the evaporation flux, perpendicular to the electroosmotic flux and related to the surface area. As can be seem, it is approximately constant and is, as expected due to low temperatures, much smaller than the electro-osmostic flux (0.14 $cm \cdot d^{-1}$). As in most soil remediation setups at the bench-scale, gravity flux is prevented by the walls of the electrochemical cell and hence it is not considered. Changes produced by this flux are one of the key points that are evaluated in scale-up processes [32]. Evaporation process has important influence in the electrokinetic and also in the biological treatments. On one hand, electrokinetic treatment is only possible in saturated or semi-saturated soils, in which ionic and electrical conductivity are ensured. On the other hand, biological degradation is carried up in the water contained in the soil, so the process is inhibited when the soil is dried. Electroosmotic transport process has decisive influence on the transport of water soluble species throughout the soil matrix. In this case of study, as it is discussed below, this fact is directly related with the dispersion of the diesel pollutant with the surfactant in the water contained in the pores of the soil. Hence the importance of taking into account all the different process that influence in the water content of the system.

Figure 4 shows the changes in the concentration of inorganic nutrients (nitrate, ammonium and phosphate) in the anodic and in the cathodic chambers. As can be observed, there is a significant transport of nutrients from the soil to both electrodes. This transport has to be explained in terms of two main mechanisms:

- Electro-migration of ions that explains nitrate and phosphate concentrates in the anodic well due to the opposite charge of the electrode.
- Drag by electroosmotic flux that explains the occurrence of both anions in the cathodic well as this flux is from the anodic to the cathodic wells.

This transport of nutrients has important influence on the rate of the biological degradation process. Electrokinetic transport of the different elements involved in the biodegradation process increase the possibilities of interaction between them. However, as it is previously commented in the introduction section, the rate of these transport processes usually is several orders of magnitude different depending on the specie transported. This may result in an inhibition of the microbial metabolic process because the removal of nutrients at higher rate at which microorganisms are transport throughout the soil. Dispersion of data presented in the Figure 4 (especially important in the cathodic well for phosphate) may be explained in terms of the many processes that affect the transport of this ions, including pH (which can precipitate the phosphates in the soil), biological oxidation (which transforms phosphates in organic phosphate and reverses this reaction), and different directions of the electro-migration and electroosmotic fluxes.

SDS and diesel are also transported from the soil to the electrolyte wells during the electrochemical treatment of the soil. This transport can be clearly observed in Figure 5 where changes during the progress of the electro-remediation in the concentration of both species are plotted. In this case, beside the dragging effect on species of the electroosmotic flux (that explains that both species appear on the cathodic well) and the electro-migration that affects mainly to the ionic SDS (and that explain the huge decrease in the catholyte), electrophoresis of micelles SDS-diesel should be accounted because this

is the only mechanisms that can explain the presence of large amounts of diesel in the anolyte.

The study of the changes in electrolyte wells composition gives many insights about the main processes happening in the soil. However, to better understand these processes, the soil was completely characterised after treatment with a post-study analysis, in which the soil was divided into four portions (in the anode to cathode direction) and each of these portions was also divided in another four sections to study axial dispersion effects on results and performance. The results obtained are shown and discussed in the next Figures.

As can be observed in the Figure 6, the moisture content of the soil slightly decreases during treatment from an initial value of 40%. This initial high value was required to prepare the biological barrier in these experiments and during the treatment is expected to be modified by previously described fluxes (electroosmosis and evaporation). Main decreases are from near the cathode and are more uniform in that direction (low axial dispersion) while axial dispersion increases in the positions near to the anode surface.

Figure 7 shows the changes in the pH and conductivity obtained in the post-study characterisation of the soil. Regarding pH, the effect of the acidic (transport of protons produced anodically from the anode to the cathode) and basic fronts (transport of the hydroxyl ions produced cathodically from the cathode to the anode) on the portions is clearly observed and pH in regions close to the electrode are strongly acidic or alkaline. However, pH in the Bio-PRB, which is placed in the middle point of the section submitted to the treatment, is kept within a range of values compatible with microorganism survival. These results suggest that this strategy of implementing bio-electrochemical processes is very interesting because it helps to preserve the life of microorganisms, at least from one

of the more serious challenges, the abrupt change in pH. Regarding conductivity, this parameter is associated with the concentration of ions. The acidic and basic fronts have determining influence on this parameter, because the increase in the concentration of protons and hydroxyl ions and the consequent liberation or fixation of ionic species in the nearly areas to the electrodic wells. However, not only the acidic and basic fronts be considered but also the transport of ions from the soil to near the anode and cathode. This transport was observed to be important when results in the electrolytes were assessed and results (Figure 7b) show that there is an increase in the conductivity near the anode (as consequence of the influence of the acidic front and maybe a concentration of ions) and a decrease in the vicinity of the cathode, suggesting a depletion of ions in these zones and precipitation processes because the influence of the basic front.

Figure 8 shows the concentration of microorganisms (quantified as $CFU \cdot g_{Soil}^{-1}$) in each of the portions of the soil. As can be observed, there is a great modification in the population contained in the soil during treatment. Initially, microorganisms were seeded only in the biological barrier and results show that they are efficiently spread in the soil because they are present at higher concentrations in other portions. Nevertheless, axial dispersion is very high. This dispersion could be related to the very narrow range of each parameter required in each portion to support microorganism life. An interesting point is that this strategy of bio-electroremediation can support microorganism life over a large treatment time and occurrence of microorganisms is not only located at the barrier but also near the anode. Concentration in zone 1 is almost nil, which may be explained in terms of the acidic pH in this zone.

To metabolise organic pollutants, microorganisms require nutrients, therefore a lack of nutrients can be a key factor to explain low efficiencies when pH is not affecting the

growth of microorganisms. Because of this, Figure 9 focuses on nutrient concentrations. The strategy applied in this work consists of dosing initial nutrients in concentrations high enough to attain the complete removal of the pollutant. However, what can be observed is that nitrate concentration is greatly decreased during treatment and phosphate concentrations are almost completely depleted. As was previously discussed, both ions are efficiently transported to the electrolyte wells by electro-kinetic processes and hence are less available in soil portions to microorganisms for biological removal of the diesel pollutant. This can seriously affect the efficiency of the process, in particular in the case of phosphate, in which the depletion is almost complete. Nitrate can be used not only as a nutrient but also as an electron acceptor in respiration of microorganisms. Hence as it is not completely depleted, this means that microorganisms still have a nitrogen source for growth and comburent metabolic reactions. Regarding ammonium ions, they were not added to the soil but were produced during metabolic processes. The occurrence of ammonium near the cathode is almost nil because the basic pH results in the production of ammonia, which can be volatilised.

The diesel and surfactant content in the soil is presented in the Figure 10. This surfactant was added to the catholyte well and it helped to the transport of diesel to the biological degradation area of the Bio-PRB. As can be observed, it is efficiently spread to all portions of the soil because axial dispersion is low. Spread of the SDS helps to explain the mobility of the nonpolar diesel pollutant to the anode well, a process that was found to be very efficient. Regarding diesel, it was observed that it is efficiently removed from the soil because its concentration decreases by 29% after two weeks of treatment (66 $kWh \cdot m^{-3}$). However, the decrease is almost uniform in all section of the soil, suggesting that transport plays an important role.

In order to quantify the influence of the different mechanisms in the removal of nutrients from the system, mass balances were performed. Figure 11 shows the mechanisms by which the different species involved in the process have been affected by the process. It is also shown in logarithmic scales that, at the onset, the low values are very interesting for discussion. For balance checking purposes, nitrates and ammonium have been summed as total nitrogen, although the concentration of ammonium is negligible compared to that of nitrates.

As stated before, total removal of diesel is approximately 30%. This value is very interesting, especially if compared to other data previously published about the remediation of soils polluted with diesel. Thus, Moliterni et al. (2012) reported that applying an optimized biological degradation process and using a slurry system, after 11 days of treatment, 95% of diesel was removed from the polluted media, with an initial pollutant concentration of 17000 $mg \cdot kg^{-1}$ [33]. In other previous work, Salehian et al. (2012) reported that using soil washing process with surfactant solutions, after 10 pore volume, 35% of the pollutant concentration was removed from an initial pollutant concentration of 10000 ppm and 45% starting from 20000 ppm [34]. On the other hand, Lee at al. (2012) reported that using a combined treatment with a biobarrier and surfactant solution washing process, after 30 days of treatment, a removal of diesel from 6000 ± 45 $mg \cdot kg^{-1}$ to 5 $mg \cdot kg^{-1}$ was achieved [35]. Applying another electrokinetic enhanced technology, using an electro-Fenton process, after 30 days of treatment and starting from 5000 $mg \cdot kg^{-1}$ pollutant concentration, a removal of 30% of the diesel was achieved in the soil [36].It is also worth to compare results discussed in this work with other previously obtain applying the EKSF treatment for the removal of another organic pollutant from the soil. Thus, Lopez-Vizcaino et al. (2014) reported that applying an electrokinetic soil flushing process a phenanthrene pollution removal of 25% was attained with an energy

consumption of 500 $kWh \cdot m^{-3}$ after three months of treatment [32], while the process shown here attains a greater removal in two weeks using only 66 $kWh \cdot m^{-3}$. This means that the combination of bioremediation and electrokinetic processes with the strategy proposed in this manuscript seems to be an interesting and potentially effective option.

Regarding other species, as can be observed from presented results, phosphate and nitrogen concentrations are very affected by biological treatment. In fact, phosphate seems limit the process because it is depleted during treatment. At this point the ratios of diesel and SDS removal by biological processes and removal of nutrients were calculated. Theoretical oxygen demand (ThOD) for diesel is 3.4 $mg_{O_2} \cdot mg_{Diesel}^{-1}$ while ThOD for SDS is 2.0 $mg_{O_2} \cdot mg_{SDS}^{-1}$. Taking this into account, the ThOD removed biologically in this system is 25969 mg. Nitrogen and phosphorus removal are 564.1 and 109.9 mg, respectively. This means that the ratios of N/COD and P/COD are 2.17 and 0.42%, respectively, values that are below the typical ratios for sludge coming from aerobic wastewater treatment processes (approximately 8 and 0.8%, respectively) but which can be explained in terms of a combined limiting concentration of phosphates and endogenous mechanisms for regeneration of P and N. This suggests that results could be improved and this challenge will be faced in future work by this group.

Regarding SDS, the effect of the removal on the diesel is not very significant (although it is appreciable as discussed in previous figures). These results, together with others previously discussed in the literature [32], suggest that electrokinetic soil flushing using surfactants is not a good technology for the removal of diesel pollution from soils unless it is combined with other more efficient technologies, as in the case described in this work.

4. Conclusions

From this work, the following conclusions can be drawn:

1. The combination of EKSF with Bio-PRB technology is an efficient technology for the removal of diesel pollution from spiked clay soils. In short periods (two weeks), this technology attains a diesel removal rate of 30%, and energy consumption below 15% is achieved. This value is much lower than others obtained in the literature for EKSF processes.
2. Nutrients and SDS are efficiently transported in combined Bio-PRB/EKSF technology by electro-migration and by electroosmotic processes. Diesel is also transported, although the extent of the transport is not high enough to attain a significant removal by these processes.
3. Microorganisms obtained from a municipal wastewater treatment plant and mixed with soil (keeping high moisture to assure good live conditions) not only survive but also acclimate rapidly to the treatment of diesel spiked soils. They also spread efficiently towards the Bio-PRB.
4. pH and lack of nutrients are the two key factors needed to improve this technology. In the first case, because extreme pH causes the death of microorganisms. In the second case, because it limits the growth of microorganisms and hence the remediation process.

Acknowledgments

The financial support of the Spanish Government through projects CTM2010-18833 and CTM2013-45612-R is gratefully acknowledged.

References

[1] M.M. Page, C.L. Page, Electroremediation of contaminated soils, Journal of Environmental Engineering-Asce, 128 (2002) 208-219.
[2] K.R. Reddy, K. Darko-Kagya, A.Z. Al-Hamdan, Electrokinetic Remediation of Pentachlorophenol Contaminated Clay Soil, Water Air and Soil Pollution, 221 (2011) 35-44.
[3] P. Canizares, C. Saez, E. Romero-Giron, J. Garcia-Gomez, J. Lobato, M.A. Rodrigo, Electroremediation of soil by electrochemical proceses. Part 1. Fundamentals, Afinidad, 64 (2007) 663-670.
[4] P. Canizares, C. Saez, E. Romero-Giron, J. Garcia-Gomez, J. Lobato, M.A. Rodrigo, Electroremediation of soil by electrochemical proceses. Part 2. Technology, Afinidad, 64 (2007) 671-680.
[5] M. Pazos, E. Rosales, T. Alcántara, J. Gómez, M.A. Sanromán, Decontamination of soils containing PAHs by electroremediation: A review, Journal of Hazardous Materials, 177 (2010) 1-11.
[6] W. Xu, C. Wang, H. Liu, Z. Zhang, H. Sun, A laboratory feasibility study on a new electrokinetic nutrient injection pattern and bioremediation of phenanthrene in a clayey soil, Journal of Hazardous Materials, 184 (2010) 798-804.
[7] M. Lahlou, H. Harms, D. Springael, J.J. Ortega-Calvo, Influence of soil components on the transport of polycyclic aromatic hydrocarbon-degrading bacteria through saturated porous media, Environmental Science and Technology, 34 (2000) 3649-3656.
[8] J.A. Cunningham, H. Rahme, G.D. Hopkins, C. Lebron, M. Reinhard, Enhanced in situ bioremediation of BTEX-contaminated groundwater by combined injection of nitrate and sulfate, Environmental Science and Technology, 35 (2001) 1663-1670.
[9] R. Lopez-Vizcaino, C. Saez, E. Mena, J. Villasenor, P. Canizares, M.A. Rodrigo, Electro-osmotic fluxes in multi-well electro-remediation processes, Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering, 46 (2011) 1549-1557.
[10] R. López-Vizcaíno, C. Sáez, P. Cañizares, V. Navarro, M.A. Rodrigo, Influence of the type of surfactant on the mobility of flushing fluids for electro-remediation processes, Separation Science and Technology, 46 (2011) 2148-2156.
[11] M.T. Alcántara, J. Gómez, M. Pazos, M.A. Sanromán, Electrokinetic remediation of PAH mixtures from kaolin, Journal of Hazardous Materials, 179 (2010) 1156-1160.
[12] Z.-Y. Dong, W.-H. Huang, D.-F. Xing, H.-F. Zhang, Remediation of soil co-contaminated with petroleum and heavy metals by the integration of electrokinetics and biostimulation, Journal of Hazardous Materials, 260 (2013) 399-408.
[13] T. Li, S. Guo, L. Zhang, F. Li, Electro-biodegradation of the oil-contaminated soil through periodic electrode switching, in, Chengdu, 2010.
[14] J.L. Niqui-Arroyo, J.J. Ortega-Calvo, Integrating biodegradation and electroosmosis for the enhanced removal of polycyclic aromatic hydrocarbons from creosote-polluted soils, Journal of Environmental Quality, 36 (2007) 1444-1451.
[15] L.Y. Wick, L. Shi, H. Harms, Electro-bioremediation of hydrophobic organic soil-contaminants: A review of fundamental interactions, Electrochimica Acta, 52 (2007) 3441-3448.
[16] E. Mena, J. Villasenor, P. Canizares, M.A. Rodrigo, Influence of soil texture on the electrokinetic transport of diesel-degrading microorganisms, Journal of Environmental Science and Health Part a-Toxic/hazardous Substances & Environmental Engineering, 46 (2011) 914-919.
[17] E. Mena, P. Rubio, P. Canizares, J. Villasenor, M.A. Rodrigo, Electrokinetic transport of diesel-degrading microorganisms through soils of different textures using electric fields, Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering, 47 (2012) 274-279.
[18] G. Lear, M.J. Harbottle, G. Sills, C.J. Knowles, K.T. Semple, I.P. Thompson, Impact of electrokinetic remediation on microbial communities within PCP contaminated soil, Environmental Pollution, 146 (2007) 139-146.
[19] B.M. Peyton, Improved biomass distribution using pulsed injections of electron donor and acceptor, Water Research, 30 (1996) 756-758.
[20] C.A.B. Schmidt, M.C. Barbosa, M.d.S.S. de Almeida, A laboratory feasibility study on electrokinetic injection of nutrients on an organic, tropical, clayey soil, Journal of Hazardous Materials, 143 (2007) 655-661.

[21] R. López-Vizcaíno, C. Sáez, P. Cañizares, M.A. Rodrigo, The use of a combined process of surfactant-aided soil washing and coagulation for PAH-contaminated soils treatment, Separation and Purification Technology, 88 (2012) 46-51.
[22] A.B. Ribeiro, J.M. Rodriíguez-Maroto, E.P. Mateus, H. Gomes, Removal of organic contaminants from soils by an electrokinetic process: The case of atrazine. Experimental and modeling, Chemosphere, 59 (2005) 1229-1239.
[23] A.B. Ribeiro, E.P. Mateus, J.M. Rodríguez-Maroto, Removal of organic contaminants from soils by an electrokinetic process: The case of molinate and bentazone. Experimental and modeling, Separation and Purification Technology, 79 (2011) 193-203.
[24] J.L. Niqui-Arroyo, M. Bueno-Montes, R. Posada-Baquero, J.J. Ortega-Calvo, Electrokinetic enhancement of phenanthrene biodegradation in creosote-polluted clay soil, Environmental Pollution, 142 (2006) 326-332.
[25] S. Suni, E. Malinen, J. Kosonen, H. Silvennoinen, M. Romantschuk, Electrokinetically enhanced bioremediation of creosote-contaminated soil: Laboratory and field studies, Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering, 42 (2007) 277-287.
[26] R. Boopathy, Factors limiting bioremediation technologies, Bioresource Technology, 74 (2000) 63-67.
[27] S.V. Mohan, T. Kisa, T. Ohkuma, R.A. Kanaly, Y. Shimizu, Bioremediation technologies for treatment of PAH-contaminated soil and strategies to enhance process efficiency, Reviews in Environmental Science and Biotechnology, 5 (2006) 347-374.
[28] P. Morgan, R.J. Watkinson, Factors limiting the supply and efficiency of nutrient and oxygen supplements for the in situ biotreatment of contaminated soil and groundwater, Water Research, 26 (1992) 73-78.
[29] S.F. Simoni, A. Schäfer, H. Harms, A.J.B. Zehnder, Factors affecting mass transfer limited biodegradation in saturated porous media, Journal of Contaminant Hydrology, 50 (2001) 99-120.
[30] K. Hayashi, A rapid determination of sodium dodecyl sulfate with methylene blue, Analytical Biochemistry, 67 (1975) 503-506.
[31] E. Jurado, M. Fernández-Serrano, J. Núñez-Olea, G. Luzón, M. Lechuga, Simplified spectrophotometric method using methylene blue for determining anionic surfactants: Applications to the study of primary biodegradation in aerobic screening tests, Chemosphere, 65 (2006) 278-285.
[32] R. Lopez-Vizcaino, J. Alonso, P. Canizares, M.J. Leon, V. Navarro, M.A. Rodrigo, C. Saez, Electroremediation of a natural soil polluted with phenanthrene in a pilot plant, Journal of Hazardous Materials, 265 (2014) 142-150.
[33] E. Moliterni, L. Rodriguez, F.J. Fernández, J. Villaseñor, Feasibility of different bioremediation strategies for treatment of clayey and silty soils recently polluted with diesel hydrocarbons, Water, Air, and Soil Pollution, 223 (2012) 2473-2482.
[34] E. Salehian, A. Khodadadi, B. Hosseini, Remediation of diesel contaminated soils using surfactants: Coulumn study, American Journal of Environmental Sciences, 8 (2012) 352-359.
[35] Y.C. Lee, S.G. Woo, E.S. Choi, Y. Ahn, J. Park, M. Lee, J.W. Yang, Bench-scale ex situ diesel removal process using a biobarrier and surfactant flushing, Journal of Industrial and Engineering Chemistry, 18 (2012) 882-887.
[36] M. Pazos, M.T. Alcántara, E. Rosales, M.A. Sanromán, Hybrid technologies for the remediation of diesel fuel polluted soil, Chemical Engineering and Technology, 34 (2011) 2077-2082.

List of Figures Captions

Figure 1, Part a: Lab scale set-up scheme. **Part b:** Final sampling points guideline.

Figure 2: Changes in the main operation parameters during the remediation of the soil by combined EKSF- bioremediation using biological PRB. Part a) Current density (◆), temperature (■); Part b) anode pH (▲), cathode pH (◆), anode conductivity (△), cathode conductivity (◇).

Figure 3: Time-course of the main flow rates and fluxes monitored during the remediation process. Part a) Flow rate of fluid added to the anodic well (◆), flow rate of fluid mobilised by electroosmosis (▲), flow rate of fluid evaporated (◇), flow rate of fluid removed by sampling (△); Part b) Electroosmotic flux (▲), evaporate flux (◇).

Figure 4: Changes in the concentration of nutrients in the anodic and in the cathodic wells during the remediation test. Part a) Nitrate concentration in the anodic chamber (▲), nitrate concentration in the cathodic chamber (△), ammonium concentration in the anodic chamber (◆), ammonium concentration in the cathodic chamber (◇); Part b) Phosphate concentration in the anodic chamber (■), phosphate concentration in the cathodic chamber (□).

Figure 5: Changes in the concentration of diesel and SDS that arrive at the electrodic wells during the remediation test. Diesel concentration in the anodic chamber (▲), diesel concentration in the cathodic chamber (△), SDS concentration in the anodic chamber (◆), SDS concentration in the cathodic chamber (◇).

Figure 6: Moisture map of the soil after the remediation test. Upper right position (△), upper left position (▲), bottom right position (◇), bottom left position (◆).

Figure 7: pH and conductivity maps of the soil after the remediation test. Upper right position (△), upper left position (▲), bottom right position (◇), bottom left position (◆).

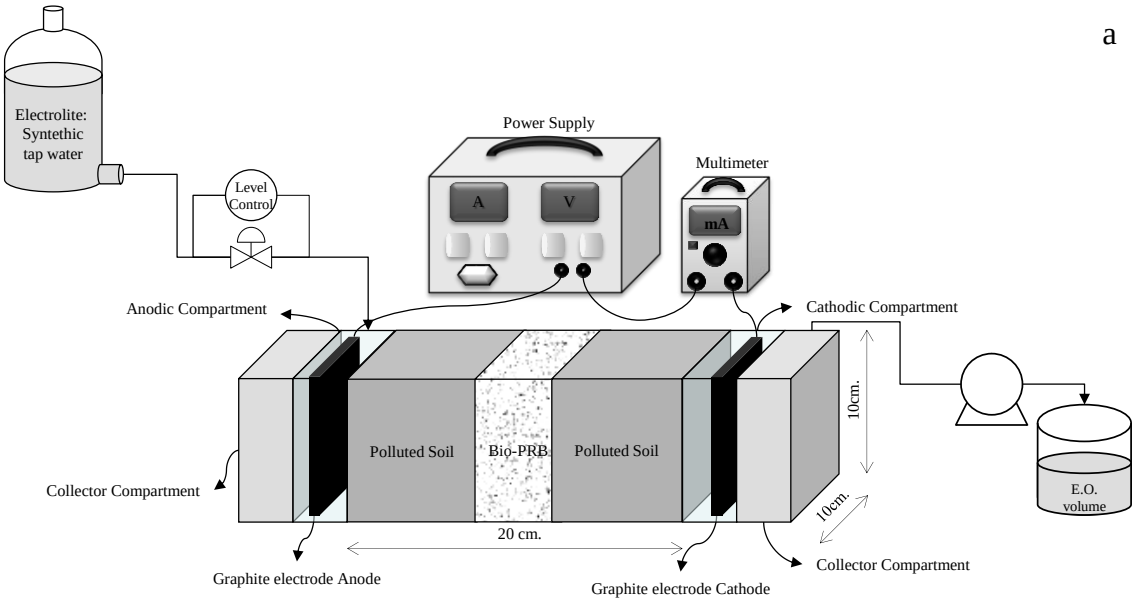
Figure 8: Microorganism concentration map of the soil after the remediation test. Upper right position (△), upper left position (▲), bottom right position (◇), bottom left position (◆).

Figure 9: Nutrient concentration map of the soil after the remediation test. Upper right position (△), upper left position (▲), bottom right position (◇), bottom left position (◆).

Figure 10: Diesel and SDS concentration map of the soil after the remediation test. Upper right position (△), upper left position (▲), bottom right position (◇), bottom left position (◆).

Figure 11: Summarized mass balance for pollutant, nitrogen and phosphorous inorganic nutrients and surfactant.

a


Electrolite: Syntethic tap water
Level Control
Power Supply
A
V
Multimeter
mA
Anodic Compartment
Cathodic Compartment
Polluted Soil
Bio-PRB
Polluted Soil
10cm.
Collector Compartment
E.O. volume
10cm.
20 cm.
Graphite electrode Anode
Graphite electrode Cathode
Collector Compartment

b

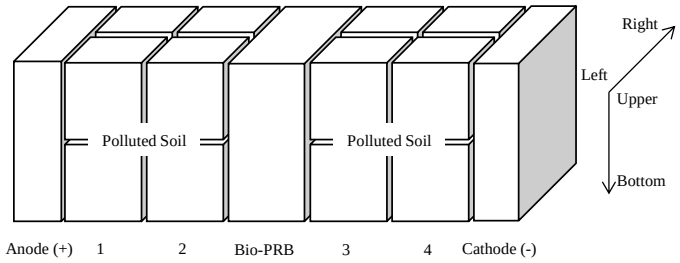

Right
Left
Upper
Bottom
Polluted Soil
Polluted Soil
Anode (+)
1
2
Bio-PRB
3
4
Cathode (-)

Figure 1

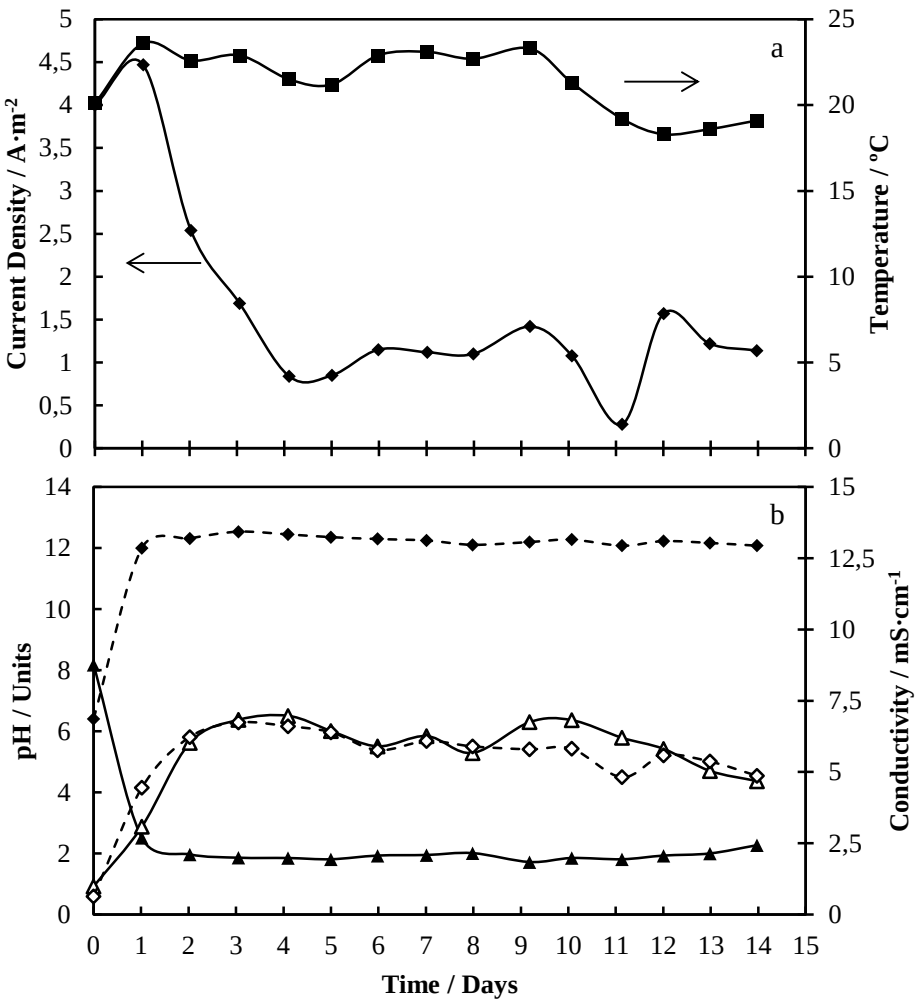

a
b
Current Density / A·m-2
Temperature / °C
pH / Units
Conductivity / mS·cm-1
Time / Days

Figure 2

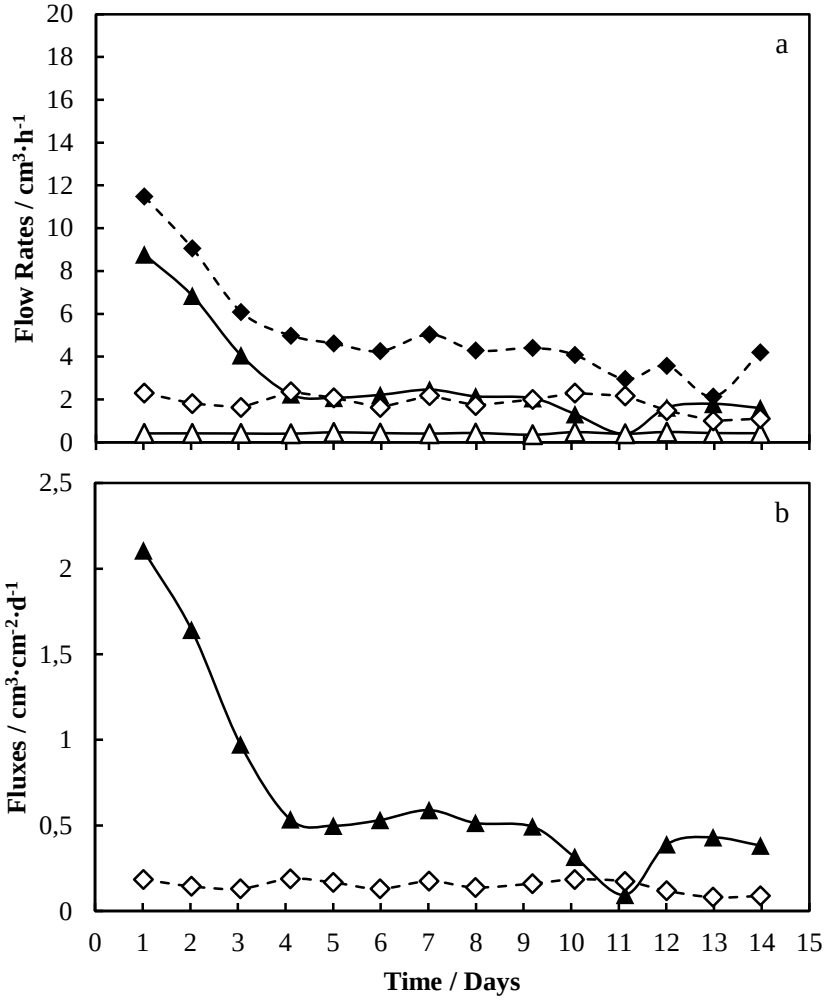

a
b
Flow Rates / cm³·h⁻¹
Fluxes / cm³·cm⁻²·d⁻¹
Time / Days
0
2
4
6
8
10
12
14
16
18
20
0,5
1
1,5
2
2,5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

Figure 3

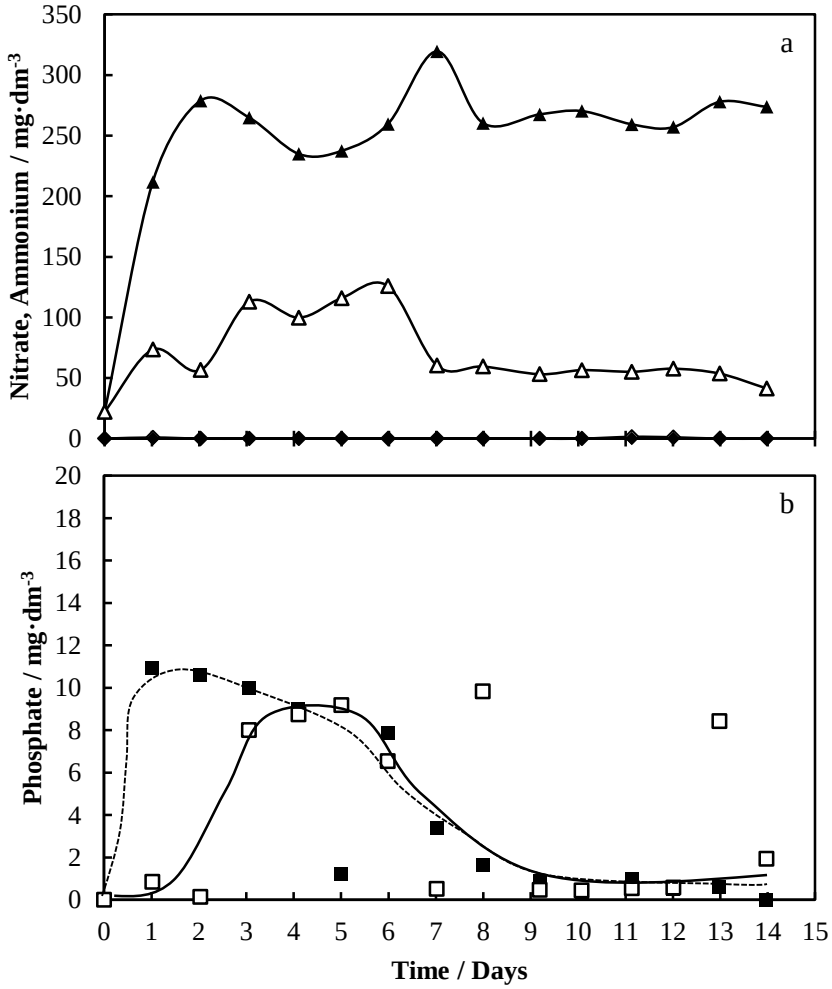

a
b
Nitrate, Ammonium / mg·dm-3
Phosphate / mg·dm-3
Time / Days
0
50
100
150
200
250
300
350
2
4
6
8
10
12
14
16
18
20
1
3
5
7
9
11
13
15

Figure 4

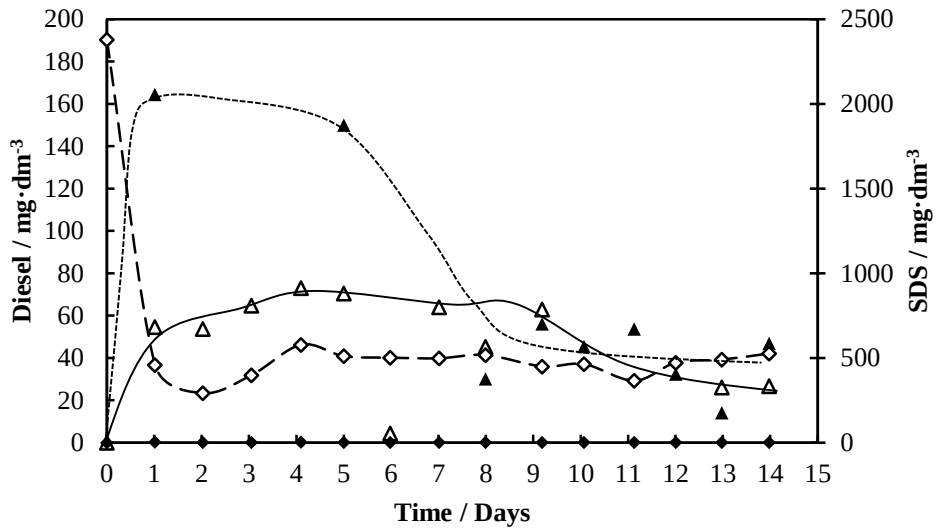

Diesel / mg·dm-3
SDS / mg·dm-3
Time / Days
200
180
160
140
120
100
80
60
40
20
0
2500
2000
1500
1000
500
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Figure 5

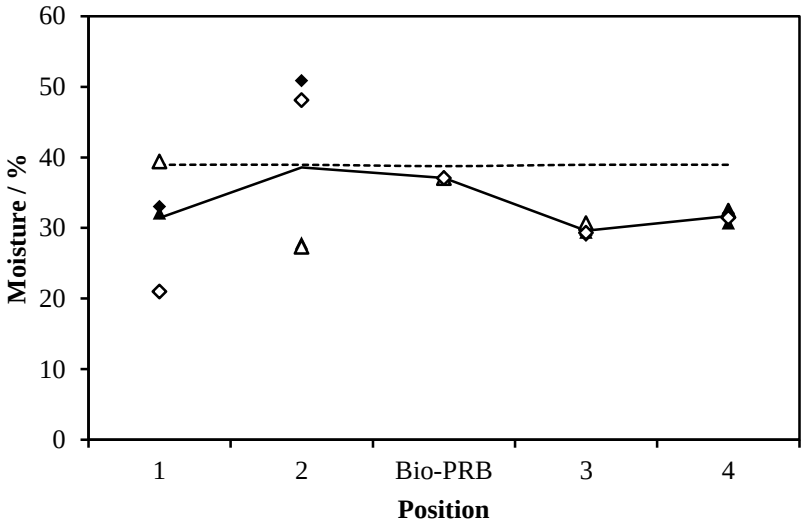


Figure 6

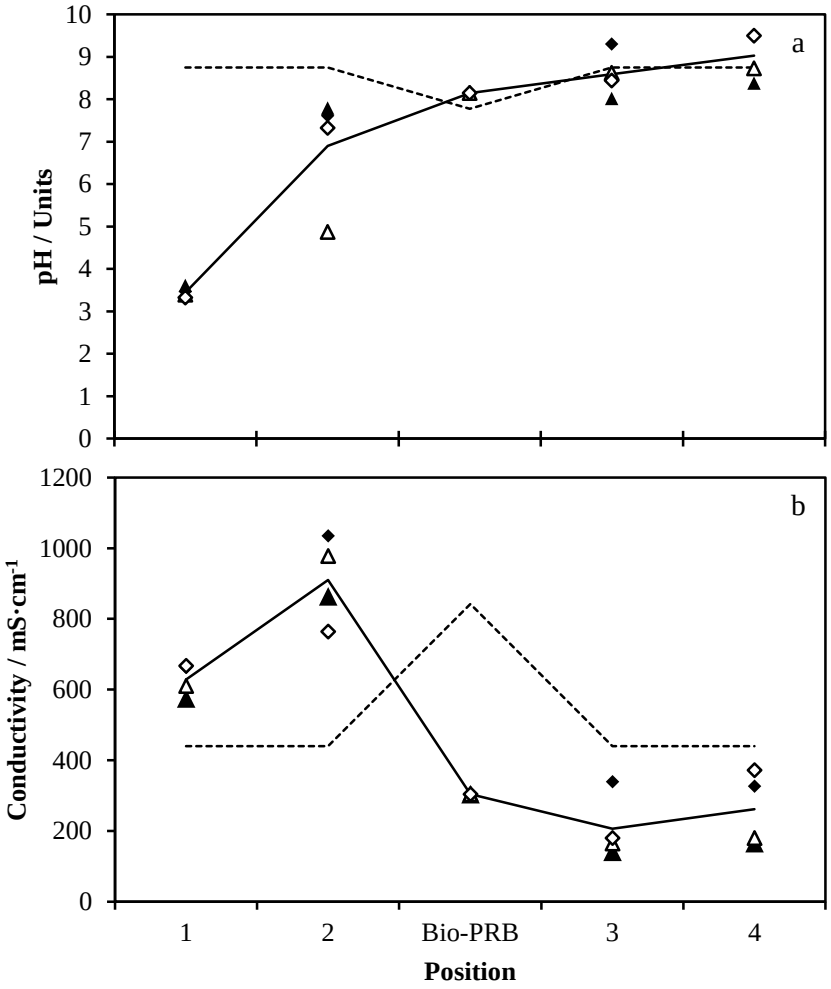

a
b
pH / Units
Conductivity / mS·cm-1
0
1
2
3
4
5
6
7
8
9
10
200
400
600
800
1000
1200
1
2
Bio-PRB
3
4
Position

Figure 7

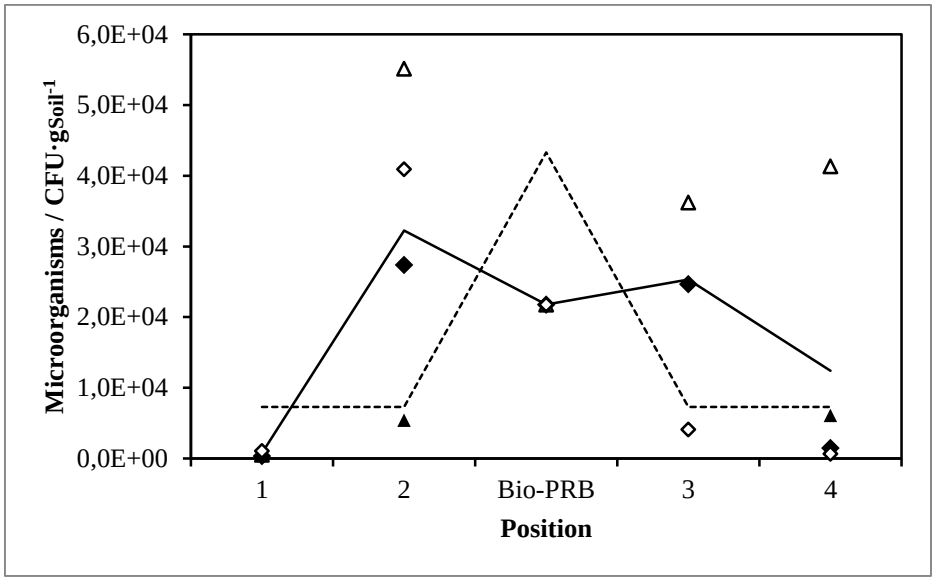

6,0E+04
5,0E+04
4,0E+04
3,0E+04
2,0E+04
1,0E+04
0,0E+00
Microorganisms / CFU·gSoil-1
1
2
Bio-PRB
3
4
Position

Figure 8

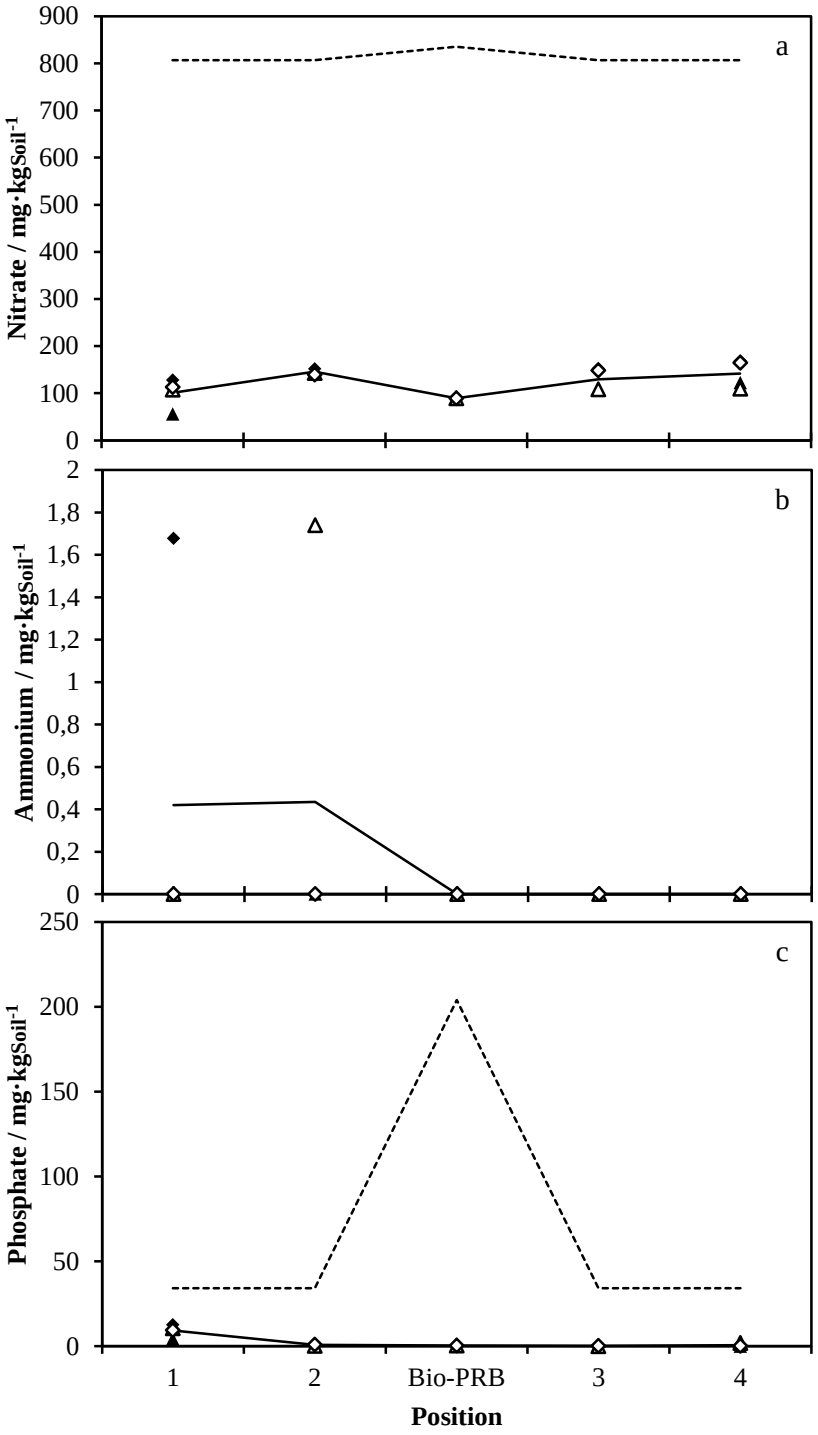

a
b
c
Nitrate / mg·kgSoil-1
Ammonium / mg·kgSoil-1
Phosphate / mg·kgSoil-1
900
800
700
600
500
400
300
200
100
0
2
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
250
200
150
100
50
0
1
2
Bio-PRB
3
4
Position

Figure 9

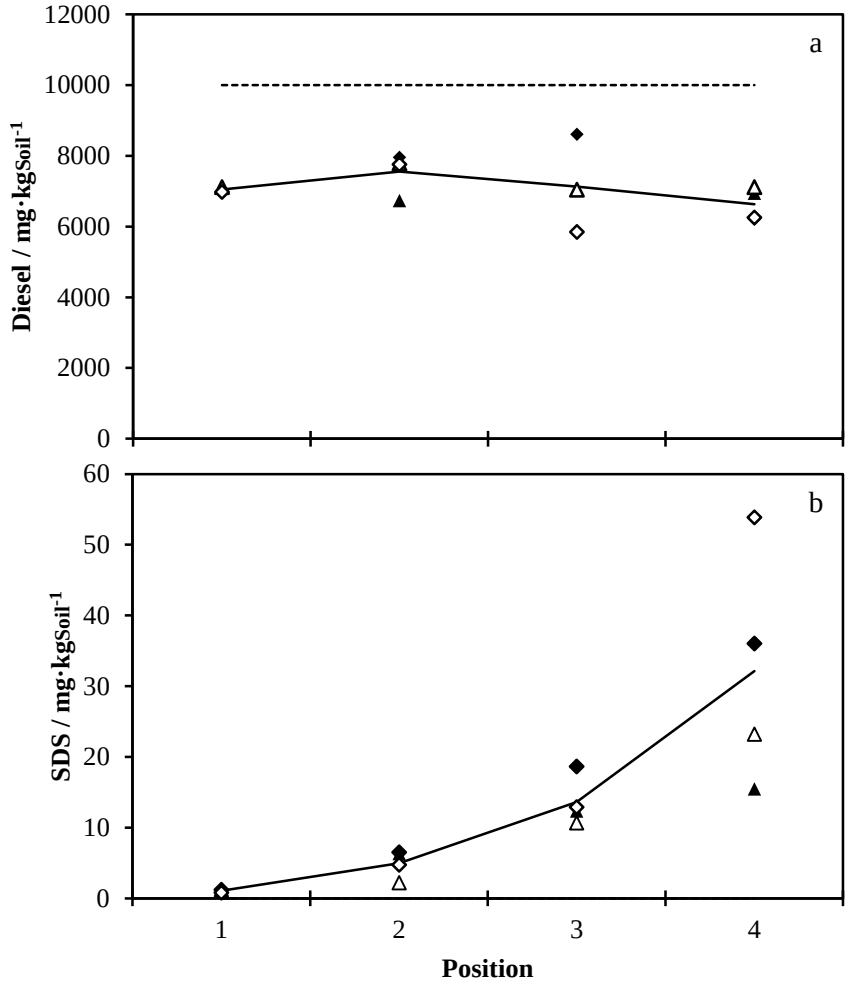

a
b
Diesel / mg·kgSoil-1
SDS / mg·kgSoil-1
Position
12000
10000
8000
6000
4000
2000
0
60
50
40
30
20
10
0
1
2
3
4

Figure 10

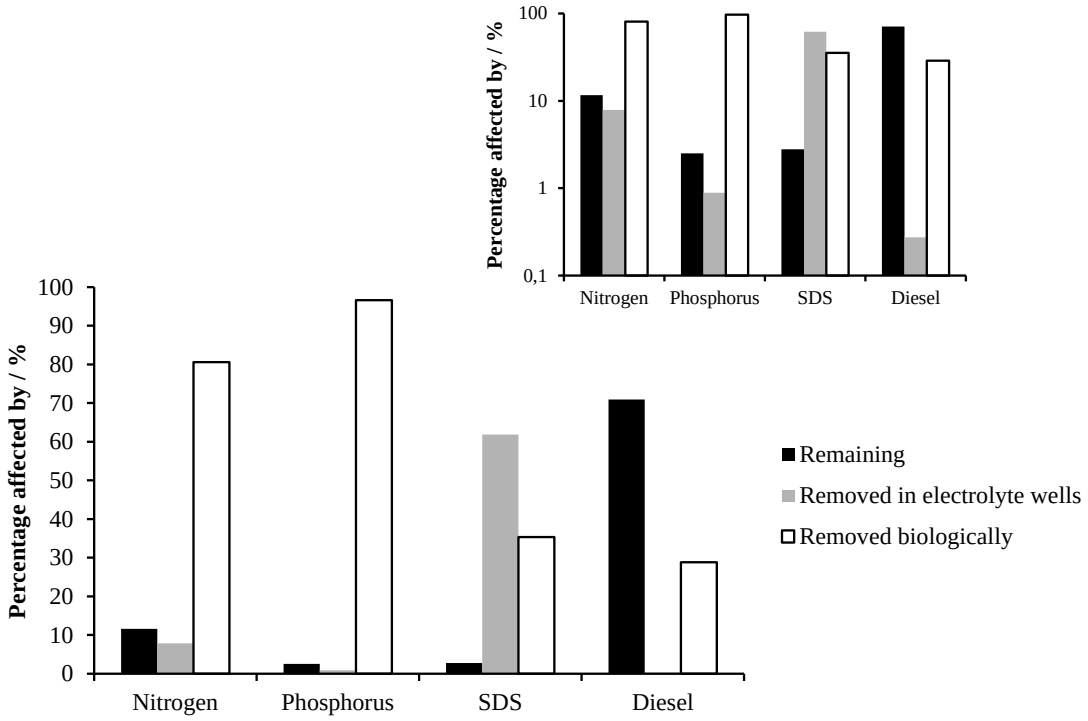

Percentage affected by / %
100
90
80
70
60
50
40
30
20
10
0
Nitrogen
Phosphorus
SDS
Diesel
Remaining
Removed in electrolyte wells
Removed biologically
Percentage affected by / %
100
10
1
0,1
Nitrogen
Phosphorus
SDS
Diesel

Figure 11

1

2

3

4