Effect of a direct electric current on the activity of a hydrocarbon-degrading microorganism culture used as the flushing liquid in soil remediation processes

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Abstract

This work aims to obtain more fundamentals for the development of novel electrokinetically enhanced bioremediation processes. It is focused on the effects of direct electric field (DC) on the microbial communities used to bioremediate soils. To evaluate this parameter, two experimental setups were used. A stirred tank reactor and a lab scale soil column. Results show that when a DC electric field (in the range 0.0-2.0 $V \cdot cm^{-1}$) is applied to a diesel-degrading microbial culture, the diesel degradation rate increases. It is also demonstrated that prolonged periods of exposure to electric fields have no significant negative effects on the viability of the microorganisms. The values of the endogenous decay constant were maintained in the range 0.02 - 0.05 h^{-1}. No significant differences were found between viability of microorganisms stressed under an electric field in a soil remediation process or in a single tank reactor.

Keywords

Electro-bioremediation, flushing fluid, diesel pollution, electric field, microbial activity

1. Introduction

Many human activities can pollute soils and groundwater. This is a complex impact due to the great diversity of pollutants (hydrocarbons, salts, metals, organic compounds, etc.) and the heterogeneity of the soil matrix. Different combinations of these two factors cause a huge variety of possible situations. Therefore, treatment technologies for the remediation of

contaminated soils (and groundwater) have recently been the subject of numerous studies. There is now a wide variety of technologies that are applicable for soil remediation [1-3].

Among these, the Electrokinetic Soil Remediation (EKSR) technology has received significant attention for the treatment of soils with low and low-medium permeability that are contaminated with organic compounds. These electroremediation techniques are based on the application of a low intensity direct electric current (DC) through pairs of inert electrodes that are inserted directly into the contaminated soil. The applied DC causes different electrokinetic processes that promote the transport of the species that are present in the soil matrix. These processes include electromigration (movement of the ions that are retained in the groundwater), electrophoresis (movement of charged particles, such as colloids) and electroosmosis (movement of the water particles that are contained in the soil pores) [4].

During the operation of the EKSR processes, a solution that maintains the water content of the soil within the acceptable limits for an electrochemical process is added. In some occasions, these fluids can also help regulate the acidic and/or basic fronts. In soil flushing processes for the remediation of hydrocarbon from soils, surfactants are also used in the formulation of these fluids. These surfactants promote the formation of emulsions between the water (polar phase) and hydrocarbon (apolar phase) molecules, thereby producing a disperse system of charged micelles that can be transported by electrophoresis through the soil to the vicinity of the electrodes. These can then be removed in the later stages of treatment [5]. Recently, the use of suspensions of microorganisms that are capable of degrading the contaminants has been proposed for the formulation of the flushing fluid, which would result in a combined bioremediation-EKSR process. In this process, the electrokinetic processes would help transport the microorganisms and the nutrients, thereby increasing the possibility of interactions between the microorganisms and the pollutant and thus increasing the remediation rate [6-11]. This new emerging technology is called electro-bioremediation.

Electro-bioremediation combines the advantages of the electrokinetic remediation technology for the treatment of occasional spills in soils with low or low-medium permeability with the advantages of bioremediation technology, which is an inexpensive and efficient technique. The usefulness of this combined technology has been demonstrated in the treatment of nitrates and organic pollutant plumes as long as the microorganisms that are used are capable of degrading the contaminants that are targeted for removal [12-22]. However, it should be noted that the application of a DC may have consequences on the contaminated soil, as well as on the microbial communities that are present in the soil and incorporated into the suspension that is used as the flushing fluid. The consequences of applying a direct electric current are

expected to depend mainly on the amperage, the treatment period, the cell type and the soil characteristics [10].

The formation of pH fronts is one of the most significant consequences that results from the application of a direct current to soil. These pH fronts are caused by the transport of protons and hydroxyl ions that are generated in the water oxidation and reduction reactions that take place on the surface of the electrodes used as the anode and cathode, respectively. The acidic front moves to the cathodes via drag, hydraulic (or electroosmotic) flux, diffusion and particularly migration. This front causes the release of pollutants that are fixed in the soil (either precipitated or retained by ion exchange) and causes a strongly acidic pH, particularly in the vicinity of the anodes, that is incompatible with microbial life. In contrast, the basic front moves towards the anode. Due to the higher mobility of the protons compared with the hydroxyl ions, which is the result of the drag and the electroosmotic flux, the acid front extends faster than the basic front. The basic front causes the fixing of pollutants in the soil (either by precipitation or ionic exchange) and the creation of an extremely basic pH in the vicinity of the cathodes that is incompatible with microbial life [19, 23-28].

Similarly, an increase in the interactions between the different elements that are involved in the pollutant biodegradation process is among the most significant consequences resulting from the application of a direct current to a microbial culture. In many studies, this consequence is described as a stimulating effect of weak direct electric currents on cell growth, glucose consumption, dehydrogenase activity and the synthesis of biopolymers [29-31]. Researchers have concluded that the use of the substrate (and thus the microbial metabolism) increases in the presence of a direct electric current due to direct (electron transfer from the electrodes to the bacteria) and indirect stimulation (electron transfer through the water electrolysis reactions) and because of the stimulation of the metabolic processes that are related to the transfer of nutrients across the cell membrane [32-35]. However, negative severe consequences have also been found due to the application of a direct current. In addition to the extreme pH values that are generated by the water electrolysis reactions in the vicinity of the electrodes, higher power values that limit the activity of the microorganisms and can even lead to their death have been described [23, 24, 36]. Other researchers have reported that the application of a direct current can induce changes in the orientation of the membrane lipids and/or the degradation of the cell wall by oxidation (anode) or reduction (cathode) [7]. In addition, the generation of toxic products can also be induced by the application of an electric field [37]. Therefore, other researchers have concluded that bacteria that have the ability to tolerate stress environments are the organisms that are most suitable for use in the flushing

liquid used in the implementation of the electro-bioremediation technique for the treatment of polluted soils [38].

The use of the electro-bioremediation technique implies the application of a direct electric current to the contaminated soil in which the microorganisms that are responsible for the degradation of the desired contaminant are present. Therefore, it is important to ensure that the modifications to the chemical and physical characteristics of the soil that are caused by the electric current do not lead to conditions under which the degradation of the contaminant by the microorganisms is not feasible. Similarly, it is also very important to ensure that the electric current does not have a direct negative effect on the cells of the contaminant-degrading microbial community.

Consequently, it is crucial to assess the consequences of the application of a direct electric current on the physicochemical characteristics of the soil under treatment and on the autochthonous or even allochthonous hydrocarbon-degrading microbial communities that are used. In this context, the main objective of this work was to evaluate the effects of the application of a direct electric current on the biological activity of a water-suspended diesel-degrading microbial consortium, which could be used as the flushing liquid for the treatment of diesel-polluted soils. The influence of the direct electric current was evaluated in different situations, such as different exposure times, different voltage gradients, and differences in the availability or biodegradability of the substrate. It is expected that the results would allow the determination of the proper range of conditions that can be used to operate future electro-bioremediation processes.

2. Experimental

Two types of experiments were performed. The influence of a direct electric current on a suspended culture of diesel-degrading microorganisms was first evaluated (**Section 2.1**). The possibility of using a suspension of diesel-degrading microorganisms as the flushing liquid in an electrokinetic cell under the influence of a direct electric current was then studied (**Section 2.2**). The methodologies used for these experiments are detailed below.

2.1. Bioelectrical reactor

The experiments that were used to assess the influence of the direct current on the biological activity of the diesel-degrading culture were conducted in an installation that resembles a batch reactor with a volume of 2 litres into which the microbial culture in a growth liquid medium was introduced. Titanium plates (2 x 12 cm), which were used as inert

electrodes, were placed inside the reactor and connected to the power supply. The reactor had a magnetic stirring system to ensure the homogeneity of the medium, and a small air compressor system was connected to supply oxygen to the medium. A dissolved oxygen probe was placed inside the reactor and connected to a dissolved oxygen meter.

The experiments were started through the addition of an inoculum of the diesel-degrading microbial consortium into the liquid mineral medium, which also contained diesel oil as the sole carbon source (the microbial consortium and the growth medium are detailed in **Section 2.3**). A set of batch experiments were performed to study the effect of two variables on the biological activity of the culture: the duration of the exposure to the direct electric current and the voltage of the direct current. The exposure times used were 2 and 7 days. The voltages used were 0.0, 0.2, 0.4 and 0.6 $V \cdot cm^{-1}$. The different experiments that were performed are indicated in Table 1. At the end of each experiment, triplicate representative samples were collected (50 mL), and the biological activity of the consortium was determined through the measurement of the specific oxygen uptake rate (SOUR, $mgO_2 \cdot h^{-1} \cdot gVSS^{-1}$).

The effect of the organic substrate that was used was also considered through a new set of batch experiments. Each experiment was started through the addition of an inoculum of microorganisms into the growth liquid medium. Two different organic substrates with different biodegradability were used (diesel oil and glucose), and the initial organic substrate concentration was held constant throughout the experiments (1% v/v and 1 $g \cdot L^{-1}$, respectively). An experiment with a retention time of 5 days and no DC was first performed, during which a conventional batch substrate biodegradation process was performed. After the 5-day biodegradation period, a DC with a specific voltage was applied (six different batch experiments, each with a different electric field ranging from 0.0 to 2.0 V cm^{-1}, were performed, as indicated in Table 2). The DC was maintained for 75 h. Triplicate representative samples were collected (50 mL) throughout each 75 h experiment. A number of parameters were also measured throughout each experiment: the biomass concentration (expressed as the amount of volatile suspended solids, $mgVSS \cdot L^{-1}$), the number of viable microorganisms present in the culture (expressed as the number of colony forming units per millilitre, $CFU \cdot mL^{-1}$), and the SOUR ($mgO_2 \cdot h^{-1} \cdot gVSS^{-1}$).

2.2 Electrokinetic cell

Experiments were also performed to evaluate the possibility of using the suspension of the diesel-degrading microorganisms as the flushing liquid in the electrokinetic treatment of contaminated soil. The microorganisms can be circulated through the soil matrix by the electrokinetic transport processes. A study of the mobility of the microorganisms through soils

with low or low-medium permeability and different textures (clay and silt) as a result of the application of direct electric fields with three different voltage gradients (0.0, 1.0, 2.0 $V \cdot cm^{-1}$) was thus performed. The lab-scale glass cell consisted of two electrodic compartments (each with a volume of 200 mL) that were connected through a tubular compartment with an approximate volume of 16 mL into which the soil was loaded. The electrodes that were used in both the anode and cathode were porous graphite rods (1 x 1 x 10 cm).

The soils used in these experiments were kaolin and silt. Kaolin, which is a synthetic clay soil with an approximate particle size of 1 μm, was provided by Productos Químicos Manuel Riesgo (Spain). The silty soil, which was obtained from the banks of a river, was screened to obtain a homogeneous soil fraction with a particle size of 20 to 50 μm. The soil was then sterilised at 121ºC for 15 minutes to remove any microbial communities.

Briefly, the experimental procedure was as follows. First, the soil was wetted with a bicarbonate/carbonate (HCO_3^-/CO_3^{2-}) buffer solution (30 $g \cdot L^{-1}$ $NaHCO_3$) to ensure electrical and ionic conductivity throughout the entire circuit and to control the influence of the acidic and basic fronts on the mobility of the bacteria. The wet soil was placed in the central compartment of the experimental setup. Then, 150 mL of the medium in which the diesel-degrading microorganisms were grown, which contained approximately 10^9 colony-forming units per millilitre ($CFU \cdot mL^{-1}$) of the bicarbonate buffer, was introduced into the anodic compartment. At the same time, 150 mL of the same sterile buffer but without any microorganisms was added to the cathodic compartment. The power supply was then connected. The voltage gradients used were 0.0, 1.0 and 2.0 $V \cdot cm^{-1}$. The duration of the experiments was 50 h. The number of viable microorganisms in the anodic and cathodic compartments was measured throughout the experiments by sampling and measuring the $CFU \cdot mL^{-1}$.

2.3. Diesel-degrading culture and mineral medium

The diesel-degrading microbial consortium used for the experiments was isolated from a diesel-oil contaminated soil, which 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 as the mineral medium (the composition of the medium per litre of deionised water was 0.2 $g \cdot L^{-1}$ $MgSO_4$, 0.02 $g \cdot L^{-1}$ $CaCl_2$, 1.0 $g \cdot L^{-1}$ KH_2PO_4, 1.0 $g \cdot L^{-1}$ $(NH_4)_2HPO_4$, 0.05 $g \cdot L^{-1}$ $FeCl_3$ and 1.0 $g \cdot L^{-1}$ KNO_3) and diesel hydrocarbon as the sole carbon source (1% v/v). After the enrichment process, the consortium was characterised kinetically. In addition, some of the microorganisms in the consortium were identified, including *Staphylococcus lentus*, *Stenotrophomonas maltophilia* and *Pseudomonas fluorescens*, as

reported in a previous work [39]. According to previous studies, these species are commonly found in hydrocarbon-polluted soils.

2.4. Analysis

The biomass concentration was expressed as the concentration of volatile suspended solids ($mgVSS \cdot L^{-1}$) because these two parameters are directly related. Briefly, an aliquot of 20 mL was filtrated using a fibreglass filter, dried at 105ºC for 24 h and calcined at 545ºC for 2 h. Using the difference in the weight of the dried and the calcined sample, the VSS concentration could be determined.

The concentration of the viable microorganisms ($CFU \cdot mL^{-1}$) was determined through plating on Petri dishes. These dishes were prepared using a solution containing Luria Bertani nutrient medium (with the following composition per litre of deionised water: 10 g NaCl, 5 g yeast extract and 10 g casein peptone), 15 $g \cdot L^{-1}$ of European Bacteriological Agar as the solidifying reagent, and 2 $g \cdot L^{-1}$ of glucose as the carbon source. Each dish was inoculated with 100 µL of the sample, and Digralsky handles were used to spread the sample evenly. The inoculated Petri dishes were incubated at 26ºC for 48 h, which is the time required for the colonies to grow sufficiently such that they can be enumerated.

The measurements of the oxygen consumption rate ($mgO_2 \cdot L^{-1} \cdot h^{-1}$) were performed using the dissolved oxygen meter (a Strathtox respirometer, Strathkelvin Instruments, Scotland), which allowed the determination of the variation in the dissolved oxygen concentration in the culture medium over time. The SOUR ($mgO_2 \cdot h^{-1} \cdot gVSS^{-1}$) was calculated using the VSS concentration data. For aerobic cultures of microorganisms, SOUR is known to be strongly related to the substrate consumption because it quantifies the oxygen required for its oxidation. This means that it is a good parameter to monitor substrate available for consumption at a given moment. Likewise it also gives additional information because this measurements account not only for external substrate (diesel or glucose in this work) but it also indicates the use of endogenous substrate.

It is important to note that all of the experiments were performed in triplicate to ensure the accuracy of the results that are presented in this paper.

3. Results and Discussion

Figure 1 shows the biological activity, which is expressed as the SOUR, in the microbial suspension after its exposure to a direct electric field. Different voltages and different exposure times (Figure 1a corresponds to an exposure time of 2 days, and Figure 1b

corresponds to an exposure time of 7 days) were used according to the experimental procedure and the experiments specified in Table 1. As can be observed, an electric field of up to 0.6 $V \cdot cm^{-1}$ has a positive effect on the microbial activity because the oxygen uptake rate significantly increases with an increase in this parameter.

Compared with the reference experiment (0.0 $V \cdot cm^{-1}$), the application of an electric field of 0.2 $V \cdot cm^{-1}$ for 48 h causes the SOUR response to rise by 150% (from 0.23 ± 0.09 $mgO_2 \cdot h^{-1} \cdot gVSS^{-1}$ at 0.0 $V \cdot cm^{-1}$ to 0.54 ± 0.44 $mgO_2 \cdot h^{-1} \cdot gVSS^{-1}$ at 0.2 $V \cdot cm^{-1}$). Similarly, the application of electrics fields of 0.4 and 0.6 $V \cdot cm^{-1}$ for 1 week increased the SOUR response by 68 and 139%, respectively (from 0.11 ± 0.08 $mgO_2 \cdot h^{-1} \cdot gVSS^{-1}$ at 0.0 $V \cdot cm^{-1}$ to 0.19 ± 0.06 $mgO_2 \cdot h^{-1} \cdot gVSS^{-1}$ at 0.4 $V \cdot cm^{-1}$ and 0.27 ± 0.09 $mgO_2 \cdot h^{-1} \cdot gVSS^{-1}$ at 0.6 $V \cdot cm^{-1}$). Both sets of experiments clearly indicate that the application of a low voltage DC to the culture has a positive effect.

Previously reported batch experiments using the same substrate and microorganisms showed that 2 days corresponded to the exponential growth phase of the culture [39]. At this time, there are no limitations in the availability of the organic substrate. In contrast, after 7 days, the culture is in its endogenous growth phase, and the organic substrate is depleted. Consequently, the SOUR values shown in Figure 1a are higher than those observed in Figure 1b. These differences are caused by the differences in the substrate concentration. According to these results, the positive effects of the application of a DC are maintained throughout this complete biological growth process.

Once clarified that electric fields have a positive effect on the microbial activity, it was considered important to increase the range of the electric field up to the typical range of soil electro-remediation processes (0.0-2.0 V cm^{-1}) and to assess the influence of a different substrate of that to which the microorganisms are adapted, in order to check robustness. Figures 2, 3 and 4 show the main results of the experiments that are specified in Table 2. These figures show the relative variations (from the initial value) in the biomass concentration (as VSS), the concentration of viable microorganisms (as CFU), and the respiration (as SOUR) values when a direct electric current is applied to a microorganism culture that uses glucose (part a) or diesel (part b) as the carbon source. The culture media were exposed to electric fields of 0.0 (control experiment), 1.0 and 2.0 $V \cdot cm^{-1}$. As indicated in Section 2.1, a retention time of 5 days (during which no DC electric field was applied) was used before the experiments. After this period, a conventional batch substrate biodegradation process was performed.

When glucose was used as the carbon source, the VSS concentration increased rapidly within the first 10 h. After 10 h, the trend reversed and the VSS concentration started to

decrease (Figure 2a). This effect can be explained through the rapid depletion of the glucose during the first 10 h, which is accompanied by the corresponding production of microorganisms (the increase in the VSS). After 10 h, however, the endogenous phase starts (thus, the VSS concentration starts to decrease). When diesel is used as the carbon source, the substrate depletion stage extends to 48 h because of the slower degradation of diesel. This is reflected by the less pronounced increase in the VSS concentration (Figure 2b). Once the substrate is depleted at 48 h, the endogenous stage starts and the VSS concentration starts to slowly decrease. For both substrates, it was found that the application of a direct electric current had no influence on the variations in the VSS concentration that were observed. Furthermore, there are no significant differences between results obtained applying DC in the range studied. The presence of the DC does not seem to have a great influence in the variation of the SSV. The used of different organic substrates, and the different corresponding degradation rate, has a more important influence in the variation of the SSV observed than the electric currents applied to the culture media

In all cases, the concentration of viable microorganisms decreases by approximately one order of magnitude during the 75 h of monitoring, regardless of the electric field and the carbon source (Figure 3a for glucose and Figure 3b for diesel). However, when glucose is used as the carbon source, the decrease in the concentration of viable microorganisms starts after the first 10 h, which corresponds to the beginning of the endogenous phase. When diesel is used as the substrate, the decrease in the concentration of viable microorganisms starts after the 48 h. The application of a direct electric current to the biological reactor did not have a clear influence on the variations in the concentration of viable microorganisms that were observed. Taking also into account results obtained related with the metabolism of the microbial consortium the application of the electric current does not seem to have a remarkable influence in the viability of the colonies of the microbial metabolism studied, although in the case of degradation of diesel, results obtained for 1V/cm give the impression of a better performance

The variations in the respiration rate were then analysed. When glucose was used as the substrate, the SOUR remained approximately constant throughout the 75 h, regardless of the applied electric current (Figure 4a). This finding can be explained by taking into account that the culture media is in the endogenous phase during most of the experimental time and that glucose is metabolised only at the very beginning of the process, potentially after first being bio-adsorbed. However, when diesel is used as the carbon source (Figure 4b), an increase in the SOUR values is observed during the degradation of the substrate. This increase is higher with

the lower voltage gradient that was used in these experiments (1.0 $V \cdot cm^{-1}$). After the first 48 h, the SOUR starts to decrease and continues to decrease throughout the rest of the experiment to a value that is similar to the one obtained when glucose was used as the substrate.

The effect of the voltage was different in both cases. The voltage appears to have helped the degradation of the low-degradable fractions of diesel when the culture media is close to the endogenous phase and after the microorganisms had degraded the more easily assimilable fractions of the diesel oil. In contrast, when an easily biodegradable substrate is used, such as glucose, the substrate is completely depleted by the beginning of the endogenous phase; thus, the effect of the voltage is imperceptible. Therefore, it can be confirmed that, in the absence of strong pH changes, short periods of exposure at low voltages increase the rate of oxygen consumption in a liquid culture of diesel-degrading microorganisms that use diesel as the sole carbon source. This increase may be because low levels of DC can stimulate the metabolic processes related to the transfer of nutrients into the cells [7, 10, 31, 33, 34, 36]. In this case, other substrate degradation processes can be discarded because the diesel oil substrate cannot be reduced or oxidised by the titanium inert electrodes to obtain more easily degradable species [32].

To check if there are any differences in the behaviour of microorganisms when they are used in soil remediation processes an assessment in a soil column was carried out under the same range of electric fields applied. Figure 5 shows the concentration of viable microorganisms in the cathodic and anodic compartments of an electrokinetic soil remediation setup in which the culture of the microorganisms was used as the flushing liquid for the treatment of soils with low or low-medium permeability (clay and silty soils). Because the culture of microorganisms was introduced into the anodic well, the microorganisms were mobilised across the soil to the cathode due to the electrophoretic and electroosmotic drag processes [6-11]. The concentration of microorganisms in the anodic well decreases throughout the 50 h experiments as the concentration in the cathodic well increases. However, this increase in the cathodic well is several orders of magnitude smaller than the decrease that is observed in the anodic compartment. This finding suggests that a significant contribution to the decrease that is observed in the anodic well is due to microorganism decay.

In view of these results, it can be concluded that prolonged periods of exposure to electric fields in the range 0.0-2.0 V cm^{-1} do not have significant negative effects on the viability of the microorganisms that were used in this study. These results are consistent with those obtained in previous experiments.

Based on these results, a mathematical adjustment to the variation in the concentration of microorganisms over time (Equation 1) was proposed to obtain an apparent endogenous decay constant (*k*).

$$\ln (CFU/CFU_0) = -k \cdot t \qquad \text{Equation 1}$$

Figure 6 shows the values of the kinetic constants that were obtained using the proposed model as a function of the values of the direct electric current that were applied. In most cases, the values of the correlation coefficients obtained with this model are greater than 0.9 (data not shown).

As it can be observed, values of the kinetic constant are maintained within the range 0.02- 0.05 h^{-1} for the range of electric fields 0.0-2.0 V cm^{-1}. In the case of the experiments made in the tank reactor and with diesel as substrate, endogenous decay constant remains almost invariable for the whole range of electric fields. The increase in k observed for the culture of microorganisms fed in the tank reactor with glucose can be explained by the faster assimilation of glucose and the shift of the endogenous phase down to smaller operation times. Opposite to the behaviour observed for the microorganisms contained in the tank reactor, there is a significant increase in the k value for the case of the soil remediation tests with the application of an electric field. This increase can be explained in terms of the mobility of microorganisms into the soil column. To understand this, it is important to take in mind that model proposed does not discriminate between microorganisms decay and microorganisms transport, because of its simplicity. Likewise, to clarify this point, two additional experiments were carried out at higher electric fields (3.0 and 4.0 V cm^{-1}) in both experimental setups fed with diesel. Results are compared in the onset of the Figure. It can be observed that there is a great difference in the fitting constants at higher electric fields: no change in the k for the bioreactor and a very significant increase in the case of the clay soil. These results could only be explained by an increase in the mobility of microorganisms rather than by an effect of the electric field, in spite of using very large electric fields. In addition, it has to be pointed that differences in the values of the k for each culture reflect the variability in the microorganisms contained in each culture, in spite of being produced by the same procedure. In spite of this variability, effects of electric field are clear and reproducible (all samples were done in triplicate).

4. Conclusions

In view of the results presented, it can be concluded that the application of a DC electric fields (in the range 0.0 to 2.0 V cm^{-1}) to a diesel-degrading microbial community appears to improve the degradation of diesel, promoting the microbial activity. The voltage helps the

degradation of the low-degradable fractions of diesel when the culture media is close to the endogenous phase and after the microorganisms had degraded the more easily assimilable fractions of the diesel oil. In contrast, when an easily biodegradable substrate is used, such as glucose, the substrate is completely depleted by the beginning of the endogenous phase; thus, the effect of the voltage is imperceptible. The electric field does not result in any significant negative consequences to the conditions of the soil and the substrate-degrading microbial community, if pH changes are prevented with bicarbonate buffers. There are no significant differences between the behaviour of the microorganisms when they are stressed with an electric field in a tank reactor or in a soil column.

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Figure captions

Figure 1. Variation in the SOUR in the diesel-degrading culture suspension after application of a DC: (a) 2 days exposure time, and (b) 7 days exposure time. Vertical bars mean the standard deviation of three experiments.

Figure 2. Relative variations in the biomass concentration using glucose (a) and diesel (b) as carbon source at 0.0 $V \cdot cm^{-1}$ (diamonds), 1.0 $V \cdot cm^{-1}$ (squares) and 2.0 $V \cdot cm^{-1}$ (triangles).

Figure 3. Relative variations in the concentration of viable microorganisms using glucose (a) and diesel (b) as carbon source at 0.0 $V \cdot cm^{-1}$ (diamonds), 1.0 $V \cdot cm^{-1}$ (squares) and 2.0 $V \cdot cm^{-1}$ (triangles).

Figure 4. Relative variations in the concentration SOUR using glucose (a) and diesel (b) as carbon source at 0.0 $V \cdot cm^{-1}$ (diamonds), 1.0 $V \cdot cm^{-1}$ (squares) and 2.0 $V \cdot cm^{-1}$ (triangles).

Figure 5. Concentration of viable microorganisms in the cathodic (open symbols) and in the anodic (filled symbols) compartments in the mobility experiments, using clay (a) and silty (b) soils. Voltage gradient: 0.0 $V \cdot cm^{-1}$ (diamonds), 1.0 $V \cdot cm^{-1}$ (squares) and 2.0 $V \cdot cm^{-1}$ (triangles).

Figure 6. Values of the endogenous death vs. voltage gradient applied. Clay soil (triangles), silty soil (circles), bioreactor with diesel (squares) and bioreactor with glucose (diamonds).Onset: results including test carried out at higher electric fields

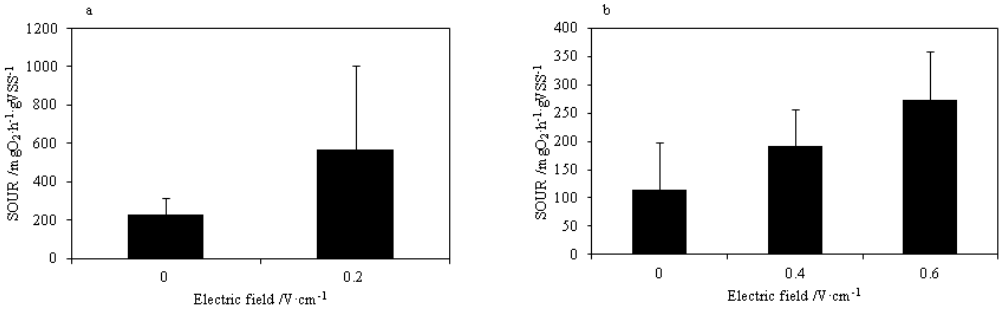

Figure 1

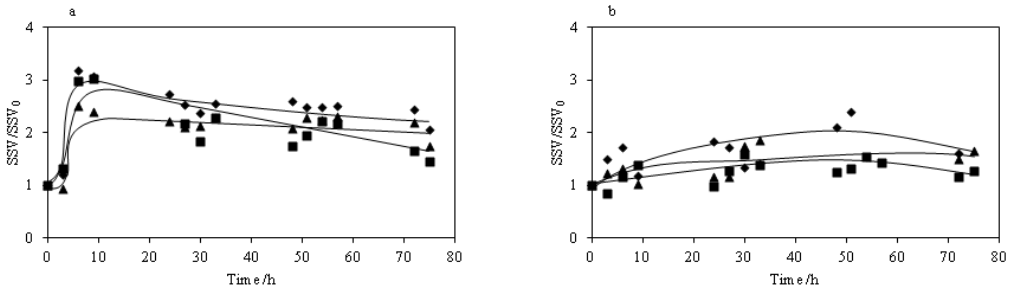

Figure 2

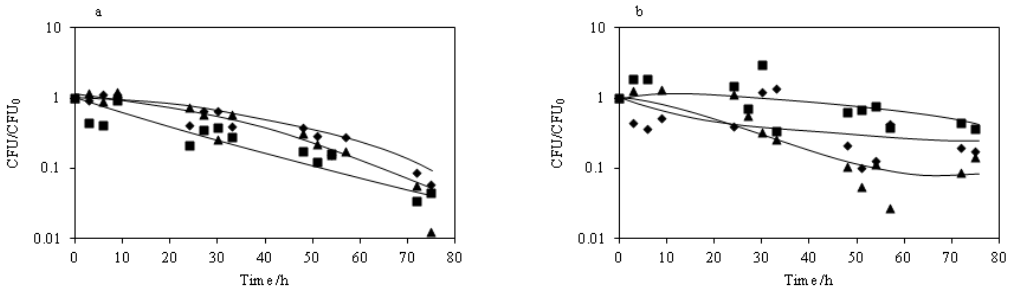

Figure 3

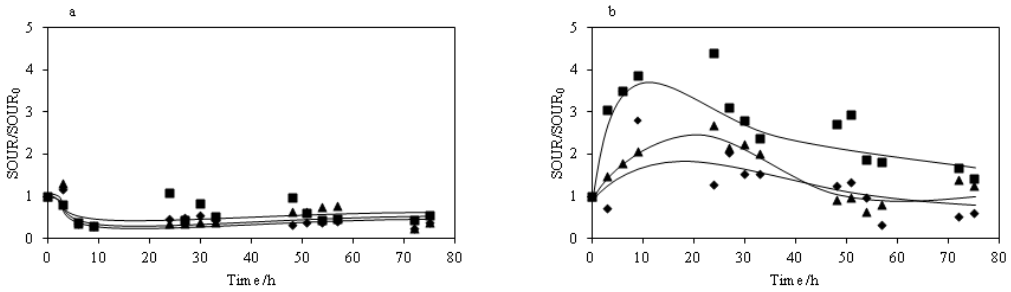

Figure 4

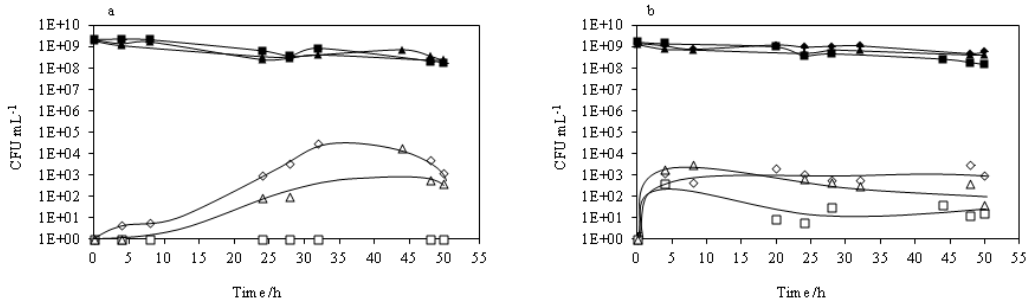

Figure 5

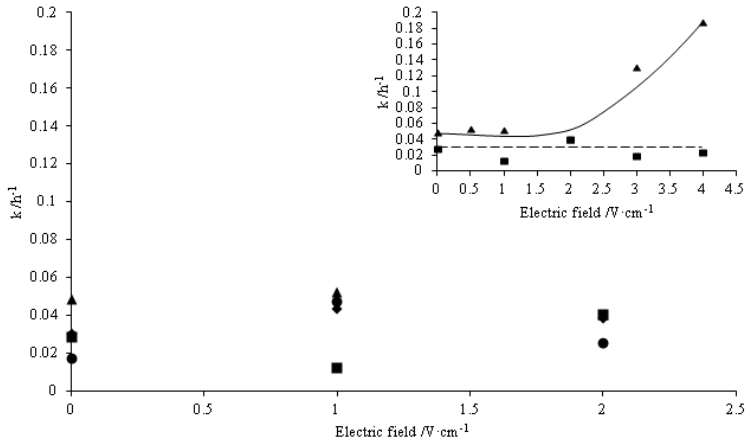

Figure 6

Table 1. Duration and voltage gradients used in the tests with low DC and diesel as carbon source.

Duration (days)	Voltage Gradient ($V \cdot cm^{-1}$)
2	0.0 - 0.2
7	0.0 - 0.4 - 0.6

Table 2. Duration and voltage gradients used in the tests performed with diesel and glucose as carbon sources.

Carbon Source	Duration (hours)	Voltage Gradient ($V \cdot cm^{-1}$)
Diesel Glucose	75	0.0 - 1.0 - 2.0