Electrokinetic transport of diesel-degrading microorganisms through soils of different textures using electric fields

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ABSTRACT

The mobilisation of diesel-degrading microorganisms in soils of three different textures (sandy, clay and silty) using electrokinetic techniques was studied. The mobilisation tests were performed using a laboratory-scale electrokinetic cell in which a synthetic soil column was inserted between the cathode and anode compartments. Microorganisms were located at the anode compartment at the beginning of each assay. A constant cell voltage was applied, and samples were taken from the cathode and anode compartments. Microbial transport through the soil strongly depended on soil particle size. Small particle sizes (silty and clay soil) travelled at low velocities (microbial transport rates of approximately 0.06 and 0.17 cm/min, respectively), while large particle sizes (sandy soil) led to high numbers of microorganisms passing through the soil column. In sandy soil, an increase in the voltage gradient did not increase the quantity of mobilised microorganisms (approximately 10^7 CFU/mL for every voltage gradient applied). For

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clay and silty soils, a higher voltage gradient led to a higher quantity of microorganisms mobilised to the cathodic compartment and a lower delay time for detecting the presence of microorganisms in the same compartment.

Keywords: Electro-bioremediation, soil texture, electric field, electrokinetic.

INTRODUCTION

Electro-bioremediation of contaminated soils is an emerging technology that combines the advantages of bioremediation and electrokinetics for the treatment of polluted soils and groundwater. It is especially recommended for the treatment of low-permeable areas polluted with nitrates and hydrophobic organic compounds (HOCs).^[1] Bioremediation is a technique based on the natural ability of microorganisms living in polluted soil to break down organic and inorganic pollutants into nutrients that the microorganisms are able to use as substrates for growth and maintenance, with or without the presence of oxygen as an electron acceptor.^[2] This technique can be used *ex situ* or *in situ*.^[2-3] *In situ* biodegradation of polluted soil or groundwater presents particular difficulties due to the varying material and energy-transport resistances in a heterogeneous medium such as the soil matrix. Electrokinetic techniques have been shown to be an efficient alternative in the treatment of low-permeability polluted soil. The use of electrokinetic phenomena can facilitate bioremediation by enhancing the mobility of pollutants, microorganisms, nutrients and water through the heterogeneous soil matrix, therefore increasing the contact between them.^[3-4]

In recent years, interest in this new technology has increased, leading to an increase in published research. Studies have investigated the mobility, viability and activity of hydrocarbons and

polycyclic aromatic hydrocarbon (PAH)-degrading bacteria in the presence of an electric field, showing that electric current is an important tool for the transport of bacteria in addition to limiting nutrients in the soil, with an efficiency that depends on the physicochemical properties of the bacteria and soil matrix. ^[3-10] There is great interest in applying electric fields to the ground to move and stimulate the degradation of pollutants, but it is also very important to understand the impact of this technique on soil microbial communities, microbial enzyme activity and soil health, which mainly depend on the intensity and duration of treatment, type of electrodes used and the medium. ^[1] Numerous studies have been conducted related to the direct impact of applying electric fields on autochthonous and allochthonous microorganisms living in the soil. ^[1,4,11-18]

The objective of this work was to study the electrokinetic transport of diesel-degrading microorganisms through model soils with different textures (sandy, clay and silty) under different voltage gradients (0, 200 and 400 V/m). Mobility experiments were performed using a laboratory-scale electrochemical device. A soil column was inserted between the cathode and anode compartments, and the microbial mobility between both compartments was monitored.

MATERIALS AND METHODS

Microbial Characterisation

A diesel-oil contaminated soil was collected from a site around an oil refinery near Ciudad Real, Spain. This soil was characterised upon arrival at the laboratory, and the consortium of microorganisms contained in the soil was isolated and maintained and enriched over several months by providing Bushnell-Hass Broth as the mineral medium and diesel hydrocarbons as

the sole carbon source. These microorganisms were characterised kinetically and stoichiometrically as described in previous batch studies.^[19-20] After a long period of successive transfers, some of the microorganisms present in the mixed consortium were identified, including *Staphylococcus lentus*, *Stenotrophomonas maltophilia* and *Pseudomonas fluorescens*. These species are commonly found in hydrocarbon-polluted soils.^[21-22]

Soil Characteristics

Experiments were performed using SiO₂ as a model sandy soil, which was provided by Panreac Chemical Products (Barcelona, Spain) in the form of granules with diameters of approximately 1 to 2 mm. Kaolin was used as a model clay soil with an approximate particle size of 1 µm and was provided by Productos Químicos Manuel Riesgo (Spain). Silty soil was obtained from the banks of a river; it was screened to obtain the fraction with a particle size of 20 to 50 µm and was calcined at 550°C for 2 hours to remove any traces of organic matter.

Electrokinetic Cell

Microbial electrokinetic transport experiments were performed in a laboratory-scale glass electrokinetic cell consisting of anode and cathode compartments, each with a volume of 200 cm³. The tubular central compartment into which the soil was loaded was 5 cm long with a capacity of 16 cm³ (Fig. 1). The electrodes used in both the anode and the cathode were porous graphite rods. Electric current was supplied by a power supply (HQ Power) with a working range of 0 to 32 V and 0 to 10 A. Because the currents to be measured were expected to be on the order of milliamps (up to 100 mA, approximately), a high-accuracy multi-meter suitable for accurately measuring currents in this range was used (Electro DH, S.A.).

Experimental Procedure and Conditions

All experiments were conducted in the installation schematised in Figure 1A. A photo of this installation is presented in Figure 1B. It has been previously determined that the direction of the movement of microorganisms is from anode to cathode.^[23] Therefore, 150 mL of the medium in which the microorganisms were growing, containing 10^9 colony-forming units per millilitre (CFU/mL), was introduced into the anodic compartment with 30 g/L of bicarbonate/carbonate ($\text{HCO}_3^-/\text{CO}_3^{2-}$) buffer. After that, 150 mL of the same $\text{HCO}_3^-/\text{CO}_3^{2-}$ sterile buffer was added to the cathodic compartment. The soil was placed in the central compartment and wetted with the same buffer solution to ensure both electrical and ionic conductivity throughout the entire circuit and to control the influence of acidic and basic fronts on the mobility of the bacteria. Finally, the power source was connected at a determinate constant voltage and the mobility of the microorganisms throughout the soil was measured using the technique explained in the next section.

Electrochemical and Microbial Measurements

Samples of 400 μL were taken from both compartments every thirty minutes over the four hours of the assays. The CFU present in the samples were counted by plating on Petri dishes.^[24] These dishes were 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/L of European Bacteriological Agar and 2 g/L of glucose as carbon source. Each dish was inoculated with 100 μL of the sample. Inoculums were spread evenly using Digrafsky handles, and the plates were incubated

for 48 hours at 26°C, which is the time required to allow for the enumeration of the individual colonies present in each sample.

RESULTS AND DISCUSSION

The first step was to measure the superficial charge of the microorganisms, which determines the direction in which they will move. This was done using *zeta*-potential measurements of the culture medium in which the microorganisms were growing, as indicated previously.^[23] In these assays, the *zeta*-potential of the particles present in the culture medium was mostly positive in the pH range in which the microorganisms can grow, between 6 and 9. This is the pH range in which the experiments were conducted, as is shown in Figure 2.A.^[24]

The most significant electrochemical reactions that can affect the pH of the culture medium, in which the microorganisms perform their vital functions, are the water oxidation and reduction reactions (Equations 1 and 2, respectively) that occur on the electrode surfaces.^[12]

Therefore, pH in the catholyte and in the anolyte should be controlled using a buffer solution. In this case, the carbonate/bicarbonate buffer was used because this is the buffer system commonly present in the natural waters.

To determine the influence of soil texture on microbial mobility, batch experiments were performed using the procedure and the different model soils previously indicated. To determine

the influence of applied voltage, experiments were conducted with voltage gradients of 200 and 400 V/m (with a separation between electrodes of 10 cm). Control experiments without voltage (0 V) were performed for every soil tested. All experiments were performed at room temperature (~25°C). The experimental time was four hours. All experiments were done in triplicate. Average results with standard deviations are presented in Figures 2 and 3.

Figure 2.B shows the average current intensity measured for each batch experiment. This current was used primarily for the electrolytic processes of water oxidation and reduction, and its value was limited by the high resistance of the soil, which was the only variable parameter when working with the same voltage. In all cases, this value was approximately constant over the entire experimental period. Figure 2.B presents the average values with the standard deviation for each value. Current obtained in the experiments with silty soil was the highest, both using 200 and 400 V/m, and current obtained in the experiments with sandy and clay soils was approximately the same. The higher current intensity obtained working with the silty soil could be due to the nature of the soil and, in particular, to the presence of natural ionic species in this soil, which was obtained from the banks of a river. In the experiments with synthetic sandy and clay soils, the only species that provided conductivity to the soil were the carbonate and bicarbonate ions present in the buffer solution.

Figure 3 shows the time course of microbial concentrations found in the cathodic and anodic compartments, comparing results for the experiments with the same soil at 0, 200 and 400 V/m. With sandy soil (Figure 3.A), varying the applied voltage had no effect on the concentration of microorganisms measured in the cathodic compartment. The concentration of microorganisms was approximately the same in the experiments with and without voltage (about 10^7 CFU/mL). This concentration was obtained in the first five minutes of the experiment. Therefore, in this

case, it can be concluded that the quantity of microorganisms mobilised by the electrokinetic phenomena is negligible compared to the amount of microorganisms displaced by diffusion caused by the difference of concentrations between the two compartments. With silty soil (Figure 3.B), the transport of microorganisms by diffusion in the control experiment displaced a quantity of microorganisms that was under the detection limit of the method used to determine the number of CFU present in the samples. For the same soil at 200 V/m, the time required to obtain a concentration of microorganisms of about 10^1 CFU/mL was 150 minutes. However, working with the same soil at 400 V/m, the time necessary to achieve a concentration of microorganisms of approximately 10^3 CFU/mL was 30 minutes. In the clay soil experiments (Figure 3.C), the mobilisation of microorganisms by diffusion in the control experiment again displaced a quantity of microorganisms under the detection limit. At 200 V/m, the time necessary to achieve a concentration of microorganisms of approximately 10^1 CFU/mL in the cathodic compartment was 180 minutes. However, at 400 V/m, the time required to obtain the same concentration was 90 minutes. It should also be noted that the quantities of microorganisms in the anodic compartment were approximately constant over the four hours of the assays. This indicates that the rate of microorganism death was negligible under the experimental conditions used and did not affect the results. The direct contact of the electrodes with the microorganisms did not cause significant cellular damage over the four hours of the experiments.

These results indicate that a higher applied voltage results in a higher quantity of mobilised microorganisms. When experiments were performed at 400 V/m, electrophoresis and electroosmosis phenomena, the electrokinetic processes that can cause mobilisation of the microorganisms, occurred at greater rates. ^[7] The transport of microorganisms was favoured in the silty soil over the clay soil. This can be easily explained in terms of the smaller pore size of

the clay soil, which favours the electroosmosis phenomena. Consideration of the electrophoretic transport of microorganisms is made more difficult because particle sizes, microorganisms and soil particles are in the same order of magnitude in the two soils. ^[4,7]

In Figure 4, the microbial transport rates observed in the different soils are presented. In the clay and silty soils without voltage, a microbial transport rate below 0.021 cm/min was observed, and the microorganisms did not move completely through the 5 cm soil portion during four hours. At a voltage gradient of 200 V/m, the microbial transport rate observed for the silty soil was slightly higher than that observed for the clay soil (0.033 and 0.028 cm/min, respectively). On the other hand, at a voltage gradient of 400 V/m, the microbial transport rate for the clay soil was 0.056 cm/min, while for the silty soil, the transport rate was 0.167 cm/min. Therefore, it can be concluded that, for the same soil, the microbial transport rate increases when the electric field applied increases. The microbial transport rate was lower for the clay soil than for the silty soil. Finally, the applied voltage did not influence the microbial transport rate in the clay soil because the microorganisms migrated to the cathodic compartment in the first five minutes of all of the experiments.

In view of these results, this emerging hybrid technology is recommended for the treatment of soil with small particles (clay or silty soil) but not for sandy soil. Sandy soil can be treated effectively using conventional “*pump and treat*” technologies. When applying these technologies, it is necessary to first analyse the most important properties of the soil, including particle size distribution, pH, buffering capacity, zeta-potential, moisture, presence of autochthonous microbial populations, nutrients and, of course, the presence of pollutants. ^[25] These parameters must be considered when applying this technology to optimise operational conditions and, above all, economic criteria.

CONCLUSION

The rate of microbial transport in soils is strongly dependent on soil particle size, and the electrokinetic transport of microorganisms is recommended for the treatment of silty and clay soils, while conventional “*pump and treat*” technologies are recommended for sandy soils. In silty and clay soils, the mobility of microorganisms increases under higher electric fields, which do not affect the microorganism death rate, at least in short term experiments. Thus, higher electric fields increase contact between pollutants and microorganisms, improving the efficiency of possible pollutant biodegradation in electro-bioremediation processes.

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307 **FIGURES CAPTIONS**

308 **Figure 1.** Experimental installation. **A)** Schematic figure. **B)** Picture of experimental setup.

309

310 **Figure 2.** **A)** pH variation. **B)** Current variation. Applied voltage 200 V/m (■ Sand, ▲ Silt, ●
311 Clay) and 400 V/m (□ Sand, △ Silt, ○ Clay).

312

313 **Figure 3.** Influence of voltage on the mobility of microorganisms in each soil. **A)** Sand. **B)** Silt.
314 **C)** Clay. Applied voltage 0 V/m (■ Anode, □ Cathode), 200 V/m (▲ Anode, △ Cathode) and
315 400 V/m (● Anode, ○ Cathode).

316

317 **Figure 4.** Influence of voltage on microbial transport rates (■ Sand, ▲ Silt, ● Clay).

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A

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Fig. 1

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B

Fig. 2

Fig. 3

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331 **Fig. 4**