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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 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.
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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

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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 l into which the microbial culture in a growth liquid medium was introduced. Titanium plates (2×12 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 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 h^{-1} 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

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

Duration (days)	Voltage gradient (V cm^{-1})
2	0.0–0.2
7	0.0–0.4–0.6

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