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Electrobioremediation of Hydrocarbon Contaminated Soil from Patagonia Argentina

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1. Introduction

Bioremediation refers to the application of biological agents, typically microbes, to the removal of pollutants from an environment (e.g. through landfarming and biopiles). The effectiveness of bioremediation depends greatly on the presence of suitable microorganisms and nutrients in the subsurface. Therefore, much remains to be done in order that a generally accepted methodology can be developed for a broad range of applications [1]. One approach has been to combine bioremediation with electrokinetics (EK) into a hybrid technology, referred to as electrobioremediation (EKB). EKB uses bioremediation to degrade hydrocarbon contaminants and EK to mobilise them. EK mobilisation of the hydrocarbon products increases their bioavailability, thereby facilitating bioremediation. Whilst commonly used in the remediation of several inorganic contaminants [2,3,4,5], EK has also been successfully applied to the remediation of several soluble organic contaminants, such as phenanthrene, benzene, toluene, and phenol [6,7]. However, the efficiency of this process is severely limited when the compounds have a low solubility or bioavailability. Under these conditions, in situ flushing has the potential to improve the electrokinetically-enhanced soil-solution–hydrocarbon interaction and subsequent contaminant removal by pumping a solution directly into the subsurface of the contaminated site. Nevertheless, in situ flushing is highly dependent on the type of flushing solution employed. If the pollutant is non-ionic, it can be removed by the electroosmotic flux. However, for fine-grained soils, in which the low hydraulic permeability does not allow effective pump and treat techniques, EK remediation may be the only useful process to remove organic pollutants. Indeed, an effective hydraulic velocity of 4×10^{-7} m/s during EK treatment at 4 V/cm has been achieved in a soil sample with a hydraulic permeability of 5×10^{-10} [8]., which can be considered essentially impermeable to mechanical pumping [9].
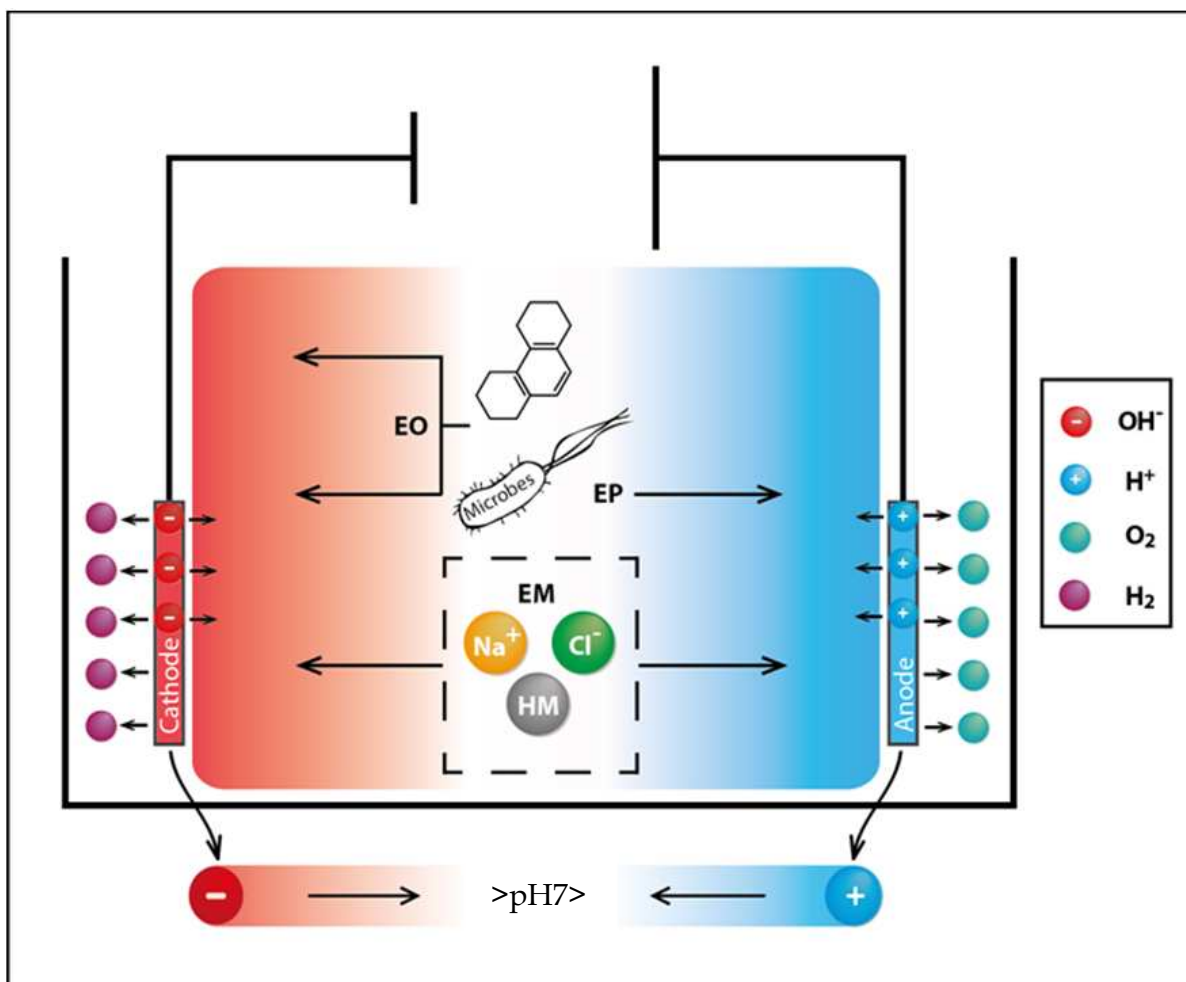
Figure 1. The effect of electrokinetic phenomena on porous soil. The application of an electric current generates hydroxide ions (OH⁻) and hydrogen gas (H₂) at the cathode and hydrogen ions (H⁺) and oxygen gas (O₂) at the anode. Subsequent diffusion of OH⁻ and H⁺ introduces a pH gradient throughout the affected subsurface that in turn facilitates electrokinetic migration of soil constituents. Microbes and PAHs (exampled with phenanthrene) migrate to the cathode by electroosmosis (EO). Electronegative microbes also migrate to the anode electrophoretically (EP). Whereas electromigration (EM) dictates the migration of ions (such as sodium and chlorine) and heavy metals (HM).

The underlying mechanism of EK involves the introduction of an electric current into soil. The introduced electric current leads to the migration of contaminants via electroosmosis, electromigration, and electrophoresis. These processes occur as a consequence of the resulting pH gradient that follows the production of hydrogen ions at the anode and hydroxyl ions at the cathode (Fig.1). These phenomena cause changes in a number of soil properties [10]. Electromigration and electrophoresis result in the movement of ions, ion complexes, and charged particles, such as colloidal clay and microorganisms toward the electrode of the opposite charge. Whereas electroosmosis arises from the migration of water towards the cathode, producing an electroosmotic flow which, in turn leads to facilitates the movement of cations, hydrocarbons and microorganisms in the direction of the fluid [11]. Accordingly, changes in the available nitrogen, phosphorus and potassium in soil were observed after EK remediation [12]. The migration of electrolytes causes an

increase in the same electroosmotic flux direction, whilst decreasing it at the opposite pole. The loss of moisture from EK-treated soil may also be due to warming by the passage of current, or exothermic reactions that may occur in the soil because the temperature increases between 1- 3 ºC [13]. Consequently, there must be a balance between electroosmotic migration, evaporation by heating or exothermic reactions and the supply of water at the anode.

The pH promotes interactions between metals and other compounds, that are a natural part of the soil, and regulates the availability of pollutants [2,10]. The passage of current directly into soil results in the electrolysis of water, thereby generating hydrogen ions in the anode and hydroxide ions at the cathode. This process occurs according to the following equations:

Cathode (reduction):

$$2H_2O + 2e^- \rightarrow 2OH^- + H_2$$
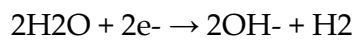
$$E° = -0,83V \text{ (alkaline)}$$

Anode (oxidation):

$$H_2O \rightarrow 2H^+ + \tfrac{1}{2} O_2 + 2e^-$$
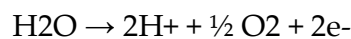
$$E° = +1,23V \text{ (acid)}$$

As a result of these reactions, an acid front and a basic front are created at the anode and cathode, respectively [14]. The ideal situation occurs when the contaminant remains are dissolved in the water and not precipitated by changes in pH, when there are no changes by contact with electrodes or interactions between the contaminant and soil particles. This situation is partially fulfilled by heavy metals and some organic compounds, such as phenols or other electrically charged compounds. However, these conditions are not met by hydrocarbons present in oil as they generally have no electric charge or, if they have, it is of very low intensity. Therefore, these hydrocarbons are normally adsorbed on soil particles and are sparingly soluble in water. Under these conditions, electroosmosis is important because it allows the migration of such compounds along the path of the migrating water.

The changes induced by the application of direct current into the soil have direct effects on the microbial activity in situ. Several studies have made efforts to enhance the transport of bacteria or nutrients for effective biodegradation through the application of EKB [15;16; 17; 18, 19].

When the current-intensity is measured with different soil textures, it was found that using only large or small particles was favourable, whereas a sandy clay soil was not favourable to any of the fundamental EK processes [20].

The processes pertaining to EKB are also themselves affected by moisture, pH, chemical nature of the contaminant and zeta potential (ζ) of the soil [2]. The zeta potential is the property that determines the load of a colloid as a function of the charged surface and environment in which it is located. Fully ionisable salts are not colloids, so its ζ is very small;

the ζ in most soils is negative. With increased acidity, ζ increases such that it can reach positive values [14]. The increase in ζ impacts on the electroosmotic flow. Soil characteristics as absorbency, ion exchange buffer capacity (pH) and load surface have a marked influence on the EKB. This shows successful results in clay, fine-grained and low permeability soil. Whereas sandy soils should have an impermeable structure at a reasonable depth to allow high humidity or saturation.

The outer surface of bacterial cells possess numerous chemical groups which, at pH 7 or greater, result in an overall negative surface potential [21]. It is therefore possible to speculate about bacterial movement under the influence of an electrical field. Soil pH changes generated allow the bacteria to migrate by electrophoresis into one of the electrodes. As can be seen in Fig.1, the negatively charged membrane causes bacteria to migrate in the direction of anode [15]. Whilst the rate of migration under an electric field is quick (5 cm/h) in aqueous media, it is slow in soil, falling to ~0.8 cm /h. [17]. At low pH, the bacterial membrane charge is positive and the direction of movement is modified to the cathode. This amphoteric property is due to the complexity of surface charges on the bacterial membrane which arise from the combination of acidic (phosphates, carboxylic acids and sulphates) and basic (most notably amines) chemical groups found on the membrane surface. Consequently, it is difficult to predict their performance with biophysical parameters [21]. This difficulty is compounded as some microbes possess the ability to change their surface polarity, thus affording them some flexibility with respect to their relative migration [22]. Nonetheless, bacterial behaviour in the electric field will strongly depend on the field's intensity. The application of the electric field will result in the migration of negatively charged micro-organisms toward the anode and one-dimensional flow of pore fluid from the anode to cathode [22,23]. Importantly, a current of 40 mA at a density of 0.1-0.2 mA/cm² is preferable in order to achieve a one-dimensional flow of a *Pseudomonas*-loaded pore fluid from the anode to cathode [24]. However, at a current density of 0.1-0.2 mA/cm², the pH stabilises in the range of 2-3 at the anode destroying the acid-intolerant microbial species and in the range of 8-12 at the cathode killing the base-intolerant species. [22], thereby limiting the efficacy of the EKB process. On the other hand, the application of a recirculating buffer solution and careful regulation of electrolyte concentrations was also shown to afford some control over the pH and thereby improve distribution of a *Pseudomonas* strain which resulted in a 60% degradation of diesel over an 8 day period [24]. Maintenance of soil pH, between 5-7, is therefore necessary in order to achieve the optimum degradation of contaminants by most native soil microbes [22].

Polycyclic aromatic hydrocarbons (PAHs) are a particularly important class of pollutant as they are generated by the incomplete combustion of carbon-based fuels and are ubiquitously found in tar, oil and coal deposits [25]. Consequently, they represent one of the most widespread and abundant class of pollutants. PAHs are of particular concern as members of this class have been identified as being mutagenic, teratogenic and carcinogenic [26]. Their abundance and relative resistance to evaporation [27] makes them ideal candidates as a model contaminant through which the effectiveness of remediation technologies can be assessed.

Our main objectives were to test the effect of EKB on: (i) the removal of PAHs, (ii) to determine the increase in bioaccessibility of PAHs in soil, which would suggest improved bioremediation performance, and (iii) to evaluate the resultant change in the bacterial communities. The measured parameters included the hydrogen ion concentration (pH) values, electrical potential, bacterial count and total petroleum hydrocarbon (TPH) content and these parameters were measured along the length of each soil specimen. The results were analyzed to assess the electrokinetic remedial efficiency.

2. Material and methods

The soil used in this study was taken from a Patagonian landfill (Table 1). Samples were obtained from a depth of between 20 and 50 cm. All samples were air-dried and sieved (2 mm) prior to use in order to facilitate the even packing of the electrokinetic cells and improve the sample homogeneity.

Physical analysis		Chemical analysis (ppm)	
pH	7.4	Chloride	260
Moisture (%)	1.88	Carbonate	< 1
Apparent Density (g cm$^{3-1}$)	1.19	Bicarbonate	101
Real Density (g cm$^{3-1}$)	1.56	Calcium	81
Zeta potential (ζ)	-25.30	Magnesium	< 1
		Sulfate	537
Hydrocarbon analysis (%)		Nitrite	< 0.01
Total	4.51	Nitrate	24.19
Aliphatic	51	Phosphate	32.9
Aromatics	31	Iron	< 0,05
Polar	18	Ammonium	1.9

Table 1. Physics-chemical Properties of Patagonian Soil Used in This Investigation.

2.1. Electrokinetic reactor

The electrokinetic reactor used in this study was similar to that used in previous electrokinetic research [28]. Electrokinetic experiments at constant potential were carried out using an experimental apparatus for the electrokinetic tests that consisted of three main parts: soil cells, electrode compartments and power supply. A schematic of the electrokinetic reactor, and the set ups of the electrokinetic cell, is shown in Fig. 2. The electrokinetic cells consisted of a glass cell (inner dimensions: length 58cm, depth 15cm and width 15cm) that was divided into three compartments: two electrodes (10cm x 15cm x 15cm) with phosphate buffers (pH 7.8 in anode and pH of 5.8 in the cathode) using platinum electrodes inside the buffers, and a soil compartment (30cm x 15cm x 15cm). The experiments were performed using three varieties of reactor cell design: (I) in the first design, the connections between compartments were made with a NaCl agar bird channel of 1cm in diameter during one

month; (II) in the second design, the electrodes were buried in the soil during one month; and (III) in the third design, the connections between compartments were done with a phosphate agar bird channel of 1cm of diameter during 150 days. All experiments were run using a constant electric field of 0.5 V/cm, and a control without electrical field was also carried out. Moisture was monitored on a weekly basis by a gravimeter method, and it was maintained at about 12%.
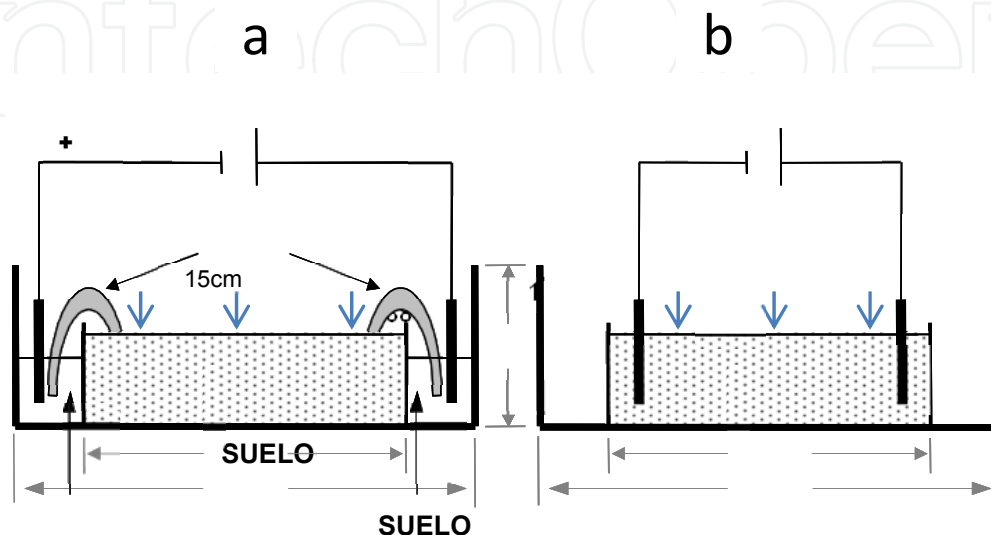

Figure 2. Design of the EK reactor used to treat polluted soil samples. Drawing A shows Experiment I, which was carried out with an agar bird channel made of Na Cl and Experiment III, which was carried out with agar bird channel made of buffer phosphate; Drawing B shows Experiment II , where the electrodes were buried in the soil.

At the end of each experiment, the soil sample was extracted from the cell and divided into 3 layers (cathode, centre and anaode), which were then divided in two samples to obtain the pH value and pollutant concentration. The pH was obtained by suspending the soil samples in de-ionised water (1:2.5, w/w) for bacterial counts, biochemical and TPH analysis.

2.2. Chemical analysis of soil samples

2.2.1. Determination of hydrocarbons via GC-analysis

Two grams of each individual sample were dissolved in 5 ml of pentane, phase separated, and percolated through 2 g of silica gel. One millilitre of the elute was carefully evaporated until dry to determine the fuel oil content of the sample. The fractions were analyzed and quantified by gas chromatography using a Varian 3800 GC, equipped with a split/splitless injector, a flame ionization detector, and a capillary column VF-5ms (30 m, 0.25 mm, 0.25 μm). The injector and detector temperatures were maintained at 200 ºC and 340 °C respectively. The Sample (1 μL) was injected in split mode and the column temperature was raised from 45 to 100 °C at a rate of 5 °C/min and a second ramp from 100 to 275 °C at a rate of 8 °C/min. The final temperature, of 275 °C, was maintained for 5 minutes.

2.2.2. Determination of TPH content by Infrared Spectroscopy (TPH-IR)

The soil TPH concentration was determined by infrared spectroscopy as previously described Environmental Protection Agency method [EPA 418.1]. Essentially, two grams of each individual sample were dissolved in 10 ml of carbon tetrachloride, phase separated, and percolated through 2 g of silica gel and the absorbance was measured at 2930 cm^{-1}.

2.2.3. Determination of TPH content by Soxhlet extraction (TPH-SE).

TPH concentration of the samples were determined by Soxhlet extractor using trichlorinethane as the extraction solvent. The extracted hydrocarbons were quantified on a mass difference basis as previously described [29] and separated into class fractions by silica gel column chromatography as formerly reported [30]. Essentially, the aliphatic, aromatic and polar oil fractions were respectively eluted using hexane (250 mL), benzene (150 mL) or 150 mL of 1:1 (v/v) chloroform-methanol.

2.3. Enumeration and isolation of aerobic bacteria.

Culturable bacteria from each sample were counted using the standard plate dilution method. One gram of soil (wet weight) was suspended in 9 ml of physiology sterile water (pH 7.2) and vortexed for 1 min at low speed. Aliquots of 100 µl of undiluted samples, and 10^{-1} to 10^{-6} dilutions were grown on TSBA (comprised of trypticase soy broth (30 g/L) and granulated agar (15 g/L)) and MBM-PGO media (comprised: NaCl (5 g/L), K_2PO_4H (0.5 g/L), $NH_4PO_4H_2$ (0.5 g/L), $(NH_4)_2SO_4$ (1 g/L), $MgSO_4$ (0.2 g/L), KNO_3 (3 g/L), $FeSO_4$ (0.05 g/L), suspended in distilled water), 30 µL of a mixture 1:1 of petroleum-diesel oil was spread on the surface once set [29] and plates incubated at 28 ºC for up to 21 days.

2.4. Chemotaxonomic analysis of soil microbe populations

The diversity of cultured sediment bacteria was determined by fatty acid methyl ester (FAME) analysis of the samples taken from the cell. FAME analysis allowed the characterization of individual bacterial colonies. Fatty acids were extracted and compared against a database, to identify isolated bacteria. From each culture plate, containing between 30 and 300 colonies, individual colonies were randomly isolated and incubated on tryptic soy broth agar for 24h. The FAMEs were extracted and analyzed by MIDI (MIDI Newark, Del., USA) as per manufacturer's instructions.

Shannon index was calculated by Sherlock (Microbial ID, version 6.0).

2.4.1. GC parameters for MIDI analysis

The MIDI microbial identification system (Microbial ID, Inc, Newark, NJ) was applied to separate fatty acid methyl ester using a gas chromatograph (HP 6890) equipped with a split/splitless injector, a flame ionization detector, a capillary column Ultra 2 (25 m, 0.2 mm, 0.33 µm); an automatic sampler; an integrator; and a program which identifies the fatty

acids (Microbial ID 6.0 version). The injector and detector temperatures were maintained at 250 °C and 300 °C respectively. The Sample (2 μL) was injected in split mode and the column temperature was raised from 170 to 270 °C at a rate of 5 °C/min.

2.5. Statistical analysis

The mean values were compared by ANOVA test by BIOM (Applied Biostatistics Inc., NY, USA). Differences were considered significant when $P<0.05$. To identify possible similarity between FAME profiles, the data were subjected to analysis of variance using PAST [31] and Sherlock (Microbial ID, version 6.0).

3. Results

3.1. Nutrient and pH control

The use of salt bridges permitted a better regulation of pH levels in the soil, especially with the use of the phosphate buffer bridge (Fig. 3) and this did affect bacterial counts (Table 2). The introduction of phosphate in the soil benefits biodegradation due to the fact that this nutrient is necessary, especially in Patagonian soils, where the concentration of nutrients is very low. On the other hand, the NaCl bridge introduced chlorine ions into the soil, the accumulation of which results in toxic effects on the bacteria [32]. This inconvenience was observed in the electrokinetic cell and arose as a consequence of electromigration produced by the applied electric field. At the end of experiment, the concentration of K^+ and Cl^- ions following their respective migration to the cathode and anode was measured. Chlorine concentrations of 1207 mg / kg were found at the cathode whereas 836 mg / kg was observed at the anode. The K^+ ions were found at a concentration of 50 and 42 mg / kg in the cathode and the anode, respectively. For experiment II, with electrode buried in the soil, significant changes in soil pH were observed throughout the cell (Fig. 3).
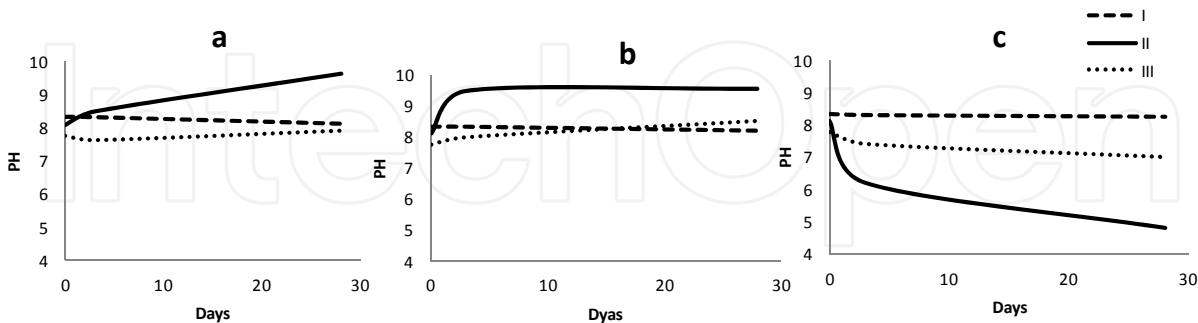

Figure 3. Effect of EKB on soil pH over time. Readings were taken from samples taken throughout the cell as follows: **a.** from the cathode, **b.** from the centre of the cell and **c.** from the anode. Data are shown for each of the reactor cell designs: I - Cells using a NaCl bridge, II – Cells in which the electrodes were placed directly into the soil and III-Cells using a PO_4^{-3} bridge.

In all three experiments, a substantial loss of moisture was observed in the electrokinetic cells. Therefore, weekly addition of water was required in order to keep the parameter

between 12 and 15%. The region near the anode tended to dry, this is why the addition of water was required in all EK cells. In addition, the electrode in the soil generated a greater change in the pH and this impacted in the bacterial communities (Table 2) as well as in the moisture values.

	CFUs/g			
	Initial	Anode	Centre	Cathode
Exp I	4.12×10^6	$6.85. \times 10^5$	2.98×10^5	2.10×10^6
Exp II	4.12×10^6	4.2×10^3	3.8×10^6	2.3×10^4
Exp III	3.10×10^7	2.77×10^7	1.62×10^8	1.04×10^8

Table 2. Obligate and Facultative Aerobic Bacteria Isolated from the EK Reactor

The results shown in Fig. 3 and Table 2 indicated that the best bridge to work with was the phosphate salt (Exp III), which could introduce nutrients to the soil and this produced an increase of bioremediation of hydrocarbons. Therefore, subsequent experiments were carried out in cells in which a phosphate bridge was placed. The nutrients introduction in Patagonian soil (Table 1), is necessary because of the soil properties [32]. In spite of this, the applied current still moves the ions. This soil needs nutrients C:N:P in the ratio 100:1:0.5 for bioremediation [32], but the anions are moved by the current, the phosphate bridges provide phosphate ions for biodegradation, and these ions accumulate in the area of the cathode.

The soil moisture contents were higher in EK remediated cells than those of the control cells due to the supply of electrolyte. However, EK remediation showed the reduced soil moisture content compared to the original soil, and the soil close to the cathode had higher moisture content than other soil, indicating the influence of electroosmosis.

Because of the electrical charge of the ions, the migration occurred and it was modified nutrient the bioavailability of phosphates. Nitrates have a relatively high mobility [33] and as shown in Table 3, the nitrates moved towards the anode. A high concentration of phosphate is seen in the cathode, probably as a result of the bridges. In accordance with previous findings [34] the values of phosphates were modified (Table 3).

	NO_3^- (ppm)	PO_4^{-3} (ppm)	NO_2^- (ppm)	NH_4^+ (ppm)
Initial	572	32.9	<0.01	1.9
Anode	1605	31.6	6.5	10.1
Cathode	479	167.7	4.1	56.7
Centre	55.9	11.4	0.96	7.6

Table 3. Electrically-Induced Migration of Nutrients in The EK Reactor.

3.2. Soil hydrocarbon content

TPH concentrations were measured by GC, TPH-IR and TPH-SE.. Analysis of the soil by TPH-IR, showed a decline in all three parts of the electrokinetics cell (Fig.4). The largest of

which occurred around the anode. After 150 days, TPH-SE showed a decrease in all parts of the cells (Fig. 4). TPH analysis showed differences in values between the hydrocarbons from cells with 0.5 V/cm and the control cell, however during the first 30 days, there was there was significant difference between the values of the cathode and the anode (P<0.05). It is at this time that nutrients are distributed in both electrodes (Table 3). Silica gel chromatography showed changes in the percentage of the fractions of the residue (Fig. 5). The aliphatic fraction showed a decrease in all three reactor treatments. The cathode showed a good degradation. The total aromatic hydrocarbons presented a better degradation in the centre of the cell. The decrease in the percentage of aliphatic and aromatic hydrocarbons was evidenced by an increase in the relative percentage of polar hydrocarbons (Fig. 5)
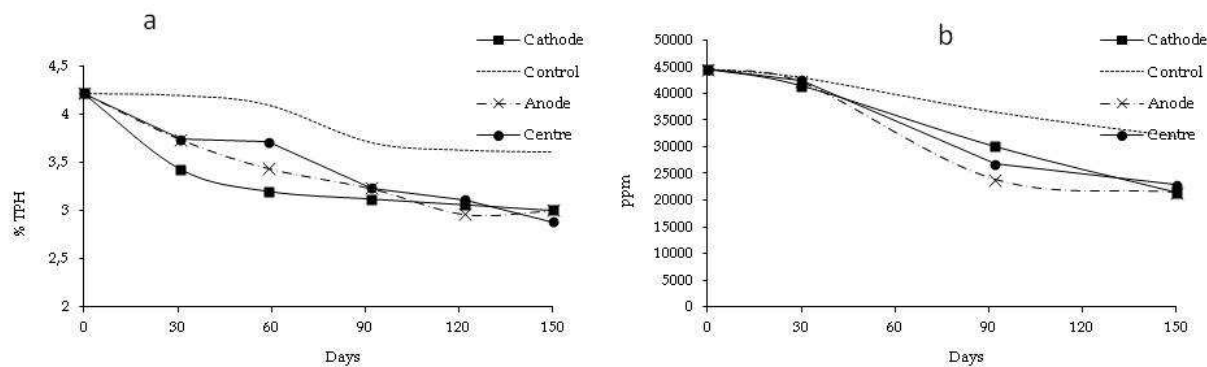

Figure 4. Effect of EKB on soil TPH levels as determined by a) TPH-SE and b)TPH-IR.
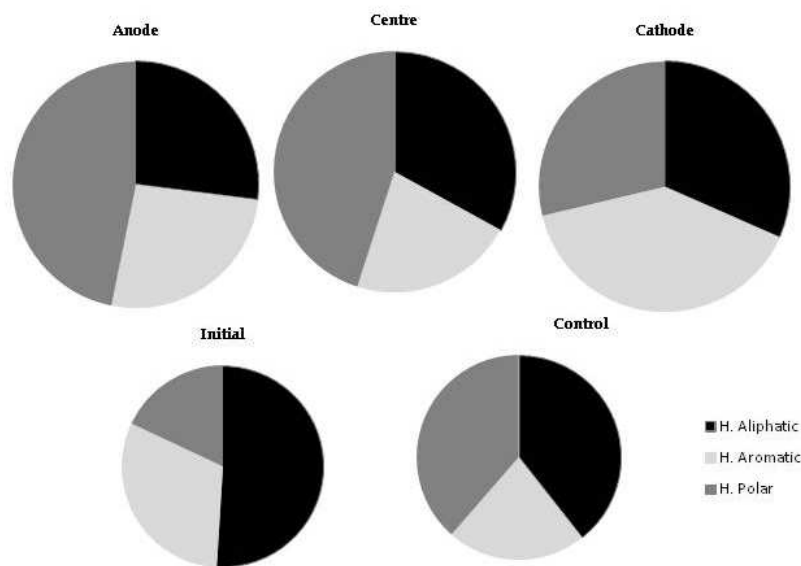

Figure 5. Effect of EKB on soil hydrocarbon content. Pie charts illustrate the relative percentages of soil hydrocarbon classes both before (initial) and, after EKB, at different locations within the cell (anode, centre and cathode).

The PAH contaminants were reduced throughout the cell, but degradation was greatest in the centre where pH was most favourable for microbial activity. Since PAHs are neutrally

charged, electromigration does not work for migration of these hydrocarbons concentration profile across the soil specimens determined at the conclusion of experiments. The results show that the PAHs were degraded preferentially in anode and centre of the cell (Table 4). The phenanthrene, fluoranthene, pyrene, benzo pyrene, chrysene, benzo fluoranthene and anthracene concentrations are relatively higher at the cathode zone than at the anode zone. Considering the initial concentration of these PAHs in the soil, significant amounts ($P<0.05$) of hydrocarbon were removed by this technique.

	Initial ppm	Anode ppm	Centre ppm	Cathode ppm	Control ppm
C11	1.629	0.000	0.000	0.000	0.000
C12	6.288	0.000	0.000	0.000	0.000
C13	4.276	0.000	0.466	0.415	0.549
C14	17.254	0.779	1.008	0.868	0.947
C15	22.979	1.534	2.036	0.000	0.418
C16	42.292	3.486	3.817	3.933	4.333
C17	63.252	5.412	4.847	7.265	15.863
C18	43.783	3.955	3.068	4.823	5.882
C19	35.130	2.819	2.016	4.174	4.844
C20	70.013	1.990	1.214	3.882	4.887
C21	68.333	2.160	1.259	11.991	2.330
C22	110.245	1.555	1.726	11.931	3.408
C23	114.417	14.697	0.000	36.278	32.746
C24	84.495	14.282	2.646	20.792	117.879
C25	59.927	6.012	0.000	56.966	23.153
C26	31.689	23.686	0.000	48.624	14.803
Pristane	0.000	0.000	0.000	0.000	0.873
2-Methylnaphthalin	0.592	0.000	0.000	0.000	0.000
Methylnaphthalin	0.680	0.000	0.000	0.000	0.000
Acenaphthylen	11.653	0.000	0.000	0.000	1.615
Acenaphthen	12.896	0.000	0.000	0.871	0.991
Fluoren	26.017	0.000	0.000	0.000	0.000
Phenanthren	38.060	0.000	0.000	1.580	2.250
Anthracen	0.000	0.000	0.000	0.919	0.808
Fluoanthen	66.422	6.406	0.000	20.744	7.081
Pyren	0.000	0.000	0.000	6.100	0.000
Benzo(a)anthracen	37.186	1.182	0.000	33.196	1.234
Chrysen	1165.801	8.570	8.277	17.502	13.579
Benzo(b)fluoranthen	9.924	0.000	0.000	0.000	6.772
Benzo(k)fluoranthen	18.451	4.484	2.989	17.166	15.328
n-Alkanes	776.003	82.368	24.103	211.943	232.041
PAHs	1387.683	20.642	11.266	98.078	49.657

Table 4. Effect of EKB on Soil Content of Hydrocarbon Compounds

3.3. Bacterial counts

Maintaining the pH values suitable for the microorganisms caused the values of the bacterial counts not to experience any modification; in all cases, the drop of a logarithm was within the error of the method (Fig. 6). The bacteria did not migrate to the area of the electrodes, as stated by other authors in the case of saturated soil [35].
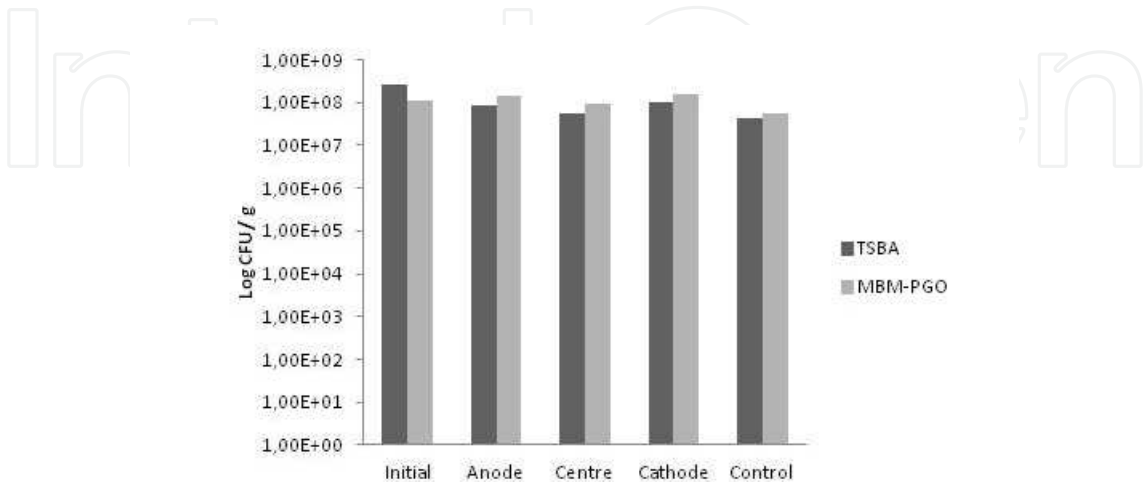

Figure 6. Bacterial count on TSBA and MBM-PGO media.

The bacterial identification was done on the bacterial count plate. In the initial sample, the genus were *Variovorax, Escherichia, Brevundimonas, Nocardia, Bordetella Mycobacterium, Rhodococcus, Acromobacter, Dierzia, Gordonia* and *Stenotrophomonas* (Fig. 7) which could grow in the agar plate in a greater number than 1 x 103. The treatment with current changed the bacterial proportion and new genus present in a small number increased their number (Fig 6).
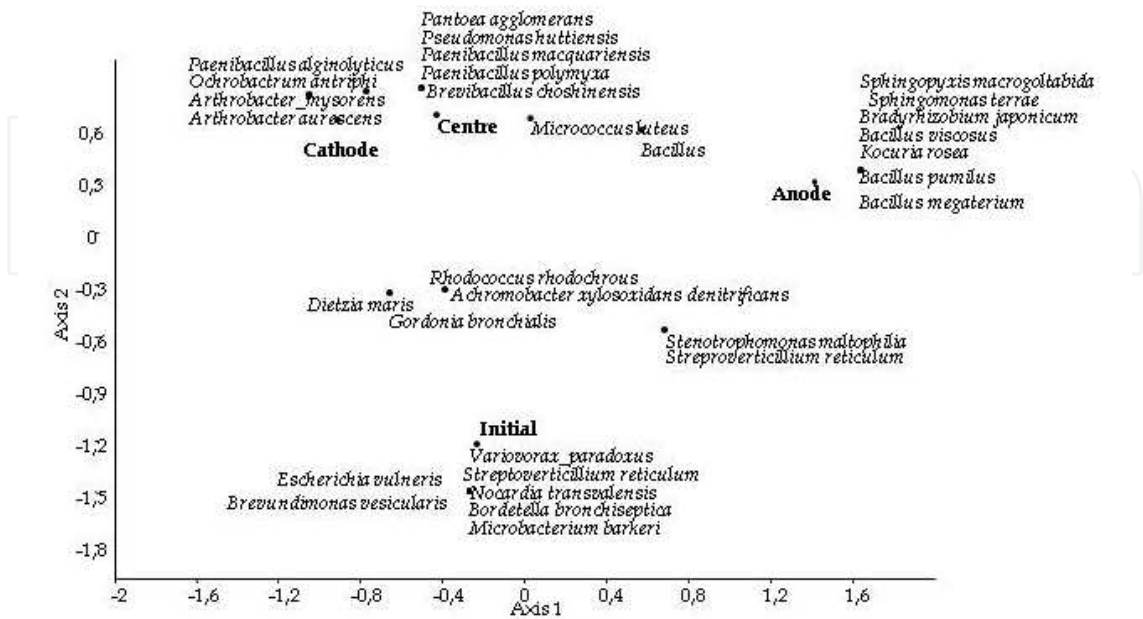

Figure 7. Statistic analysis of bacterial identification at the beginning and end of the experience.

	Initial	Cathode	Centre	Anode
Shannon_H	2.565	2.197	2.303	2.398

Table 5. Shannon indices of Soil Biodiversity

4. Discussion

The efficiency and effectiveness of electrokinetic extraction can be improved by combining the technique with other remediation technologies such as bioremediation. The results demonstrate the feasibility to degrade PAHs, a particularly problematic and toxic class of contaminant. Electrokinetics appears to be promising for a range of contaminated sites including tank bottoms which are considered dangerous waste by our legislation.

The effectiveness of the contaminant reduction during electrobioremedation depended on the bioavailability, moisture and soil pH. Among the various soil parameters, the most apparent change induced by electrobioremediation was observed in soil pH and distribution of moisture content. The bacterial number in the experiment II decreased in the anode due to the low soil pH. The soil pH is a crucial factor for microbial activity as this influences the composition and physiological characteristics of enzymes such as phosphatase, glucosidase and arylsulfatase [36]. Soil pH also affects microbial cell membrane integrity and function, and the bioavailability of nutrients and contaminants. Increased biodegradation in higher pH regions, such as cathode, has been reported for one of the PCP-degrading enzymes produced by a species of *Sphingobium* UG30 [37]. In addition, a low pH has been reported as having a major negative impact on electrobioremedation by native soil microbial communities [38,39]. Accordingly, we observed a decrease in dehydrogenase activity at locations corresponding to the acid front. In fact, in the expereince II, the soil pH changes generated by electrobioremediation were a leading cause of a decreased microbial number (Table 2). However, 0.5V/cm applied soil showed more apparent decrease in culturable bacteria compared to the soil samples of extreme initial pH [19]. This soil pH change was controlled by the use of phosphate birdge and this did not change the bacterial number between current and pH but affected the biodiversity in culturable bacteria (Fig. 7 and Table 5). The stress from the growth conditions reduces the total bacterial number and is a reason for cells entering a viable but non-culturable state [40,41]. Although electric current in EK remediation can change the bacterial membrane composition and metabolic activity, many studies revealed that weak direct current treatment has no negative effect on microbial viability and activity [42, 43, 44].

The experimental results illustrate how the application of electrokinetics to an unsaturated soil can cause major changes to the soil properties, with subsequent impact upon microbial activity and biodegradation. As expected, the lack of anodic pH control in experiment I caused progression of an acidic front through electrokinetic microcosms. However, pH control at both electrodes (experiment II) caused a large increase in moisture content in electrokinetic microcosms as the unsaturated soil absorbed water from electroosmotic flow.

In similar conditions with saturated soil this effect was not observed [18, 28, 45]. The lack of moisture change in experiment I is thought to be because the acidic pH increased the soil zeta potential, rapidly causing electroosmotic flow into the acidified region to reduce or even reverse [46]. By removing electrode fluid moisture content change was avoided but pH control had to be implemented using a regularly reversed current [47] and it may be that part of hydrocarbon fraction is eliminated in the fluids producing other contaminated residue. Changes in the concentration of pH and moisture in the soil may have mobilized pollutant fractions present in the soil in the porosity, which are often not accessible to soil microorganisms [48]. Thus, the occurrence of electroosmosis inside soil aggregates may have caused the mobilization of slowly desorbing hydrocarbons into the fast-desorbing pool, which possesses a higher bioavailability to microorganisms [49, 50]. Bioremediation in Patagonian soil with the addition of nutrients is possible; however, the remediation of PAH is problematic [29] because of their low bioavailability, which can be improved by electrobioremediation resulting in an reduction of PAH.

Maintenance of soil moisture levels proved essential, because if it decreases much it will generate problems at a metabolic level in soil bacteria. It is also necessary to apply a voltage greater than the potential in the electrodes to keep the voltage value of 0.5 V.cm^{-1} thus increasing the cost of treatment energy. By working with saturated soils, the electrolyte circulation volumes, generated by electroosmosis, are contaminated is a contaminated liquid residue. One of the benefits of our system is that unsaturated soil was not observed as a leachate product of the current application. The decrease in moisture of the tanks could also be due to factors, such as the heating system or for undesirable exothermic chemical reactions [5]. Soil temperature in electrokinetics cell remained at 24 ± 3 °C throughout the 150 days of runtime. A temperature range of 24 ± 3 °C can be considered an acceptable temperature for the biodegradation of hydrocarbons by native soil microbes. These results are in accordance with results from previous studies in which an applied voltage density of 0.3 V/cm was used for this purpose [16]. This indicates that the reduction of moisture in the anode is mainly due to the presence of electroosmotic flow in the soil.

Whilst the migration of bacteria towards the anode was expected (negative surface charge being responsible for this migration), bacteria were also found to migrate towards the cathode, which may be considered surprising at first glance. This bidirectional migration of bacteria may well have resulted from the competition between two phenomena: electrophoresis and electroosmosis [51, 52]. However, other phenomena may have contributed to the migration of bacteria to the cathode. The migration of ions and water in soil toward the cathode under the influence of the electric field is one such reason. This process could create favorable conditions for bacterial growth in the area near the cathode., although the net negative charge at their surface should make them move toward the anode. Kim et al. [53], concluded "Especially the number of culturable bacteria decreased significantly and only *Bacillus* and strains in *Bacillales* were found as culturable bacteria. It is thought that the main causes of changes in microbial activities were soil pH and direct

electric current". The results described here suggest that if soil parameters, electric potential difference, and electrolyte are suitably controlled based on the understanding of interaction between electrokinetics, contaminants, and indigenous microbial community, the application of electrokinetics can be a promising soil remediation technology when the contaminant is hard to degrade or its degradation is really slow.

When electric current is applied, different bacterial responses to changes in the physico-chemical properties, bioavailability, and toxic electrode-effect can be observed depending on the current, treatment period, cell type, and medium [14]. The soil pH had marked effects on microbial biomass, community structure, and response to substrate addition, and that low soil pH decreased microbial diversity and increased Gram-positive microbial communities such as *Bacillus* and *Arthrobacter* [38, 54] according with these authors the major presence of *Bacillus* were near the anode zone, and that bioelectrical reactors enhanced the metabolism of several strains in *Clostridium*, *Ralstonia*, *Pseudomonas*, and *Brevibacterium* [55].

5. Conclusion

These results indicate that biodegradation and electroosmosis can be successfully integrated to enhance PAH removal from soil, improved mobilisation of the less bioaccessible fraction of PAH with an electrokinetic pretreatment to reach lower residual levels through bioremediation. This process has the potential to provide an effective technology for the treatment of problematic soil. Whereas normal soil can be treated by 'classical' bioremedation. The optimization of these processes for a cost-effective application of the technology in situ to meet remediation will be the subject of future investigations.

Given the costs of this technology, we recommend this technique to Patagonian and other problematic soils which, after previous degradation, do not reach the levels prescribed by regulation. Optimization of this process for the removal of polyaromatic hydrocarbon from contaminated sites is the subject for future studies.

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