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RESEARCH ARTICLE

ELECTRO-BIOREMEDIATION OF OIL TANK BOTTOMS

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ABSTRACT

Oil tank bottoms have a significant oil concentration, which is recovered by physical and chemical methods. The waste remaining after this process can be remediated by microorganisms. In this study, the benefits of integrating electro-bioremediation, which is an hybrid technology coupling bioremediation and electro kinetics to decontaminate contaminated oil tank bottoms were evaluated in 69-day-long laboratory-scale experiments. The unsaturated oil tank bottom sample was placed in three-compartment electro-bioremediation glass cells with a potential difference of 0.5V cm $^{-1}$, applied to the electro-bioremediation cells for 69 days. Aliphatic and polyaromatichydrocarbons decreased from 80741.4 ppm to 32341.2ppm and from 414.2 ppm to 24.344 ppm respectively. The pH changed from 7.26 to 7.47 in the cathode and to 7.8 in the anode.

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INTRODUCTION

Oil tank bottomscontain a significant amount of oil that can be recovered by physical methods and incorporated into the production line. The residue from this treatment contains aliphatic and polyaromatic hydrocarbons (PAHs) of medium and high molecular weight, in a concentration that makes them unacceptable for disposal, for not complying with current legislation. In San Jorge Gulf basin, Patagonia, Argentina, hydrocarbons are degraded by microorganisms, and biopilingis a popular and sustainable approach to restore petroleum-contaminated soils (Atlas, 1991, Pucci *et al*., 2011). Electro-bioremediation, a green remediation technology developed in recent years,can promote the degradation and/or the removal of organic and metal contaminants (Niqui-Arroyo *et al*., 2006, Wick *et al*., 2007, Reddy 2010).Previous studies have shown that electrokinetic technology can remove heavy metals (Reddy *et al*., 2006), organic pollutants (Acuña *et al*., 2012), and their mixture (Maturi *et al*., 2006, Reddy *et al*., 2009) from contaminated soils.

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One problem of the electro-bioremediation technology for bacterial degradation is the pH near the electrode, which is a key factor to promote electro-osmotic flow (Page and Page2002, Kim *et al*., 1999). Controlling the pH with a buffering solution can maintain a stable pH in the soil that favors pollutant removal (Alcántara *et al*., 2012) and the addition of a few nutrients contributing to bacterial degradation (Pucci *et al*., 2012). This study aimed to evaluate the contributions of electro-bioremediation technology in the treatment of oil tank bottoms. Changes in the content of total petroleum hydrocarbons (TPH) and their components, as well as number of soil microbes and nutrients were analyzed.

MATERIALS AND METHODS

The samples were taken from oil tank bottoms located in San Jorge Gulf basin. Oil tank bottoms were subjected to a separation treatment and washing. This treatment consists of a first separation stage where the particles settle gravitational gravel and coarse sand, a second stage where the finer particles are separated from the oil mass through a process of centrifugation, and finally a wash with detergent surfactants at temperatures between 80 and 100 °C. This treatment provides three waste streams: oil, which is reinserted into the production system, water, which is reused in reinjection processes, and a sludge formed by the finer fractions of the pellet, with high oil

content that remains adsorbed in the microspores of the particles. The characteristics of the sediments are shown in in table 1. Moisture (%) 19.33%, pH7.18, apparent density 0.76, real density 1.54, porosity 0.51, organic matter 9.29 %, inorganic matter 25.71%, chloride 866.53ppm, bicarbonate 106.30ppm,calcium 338.90ppm, magnesium 0.25ppm, sulfate 77.74ppm, nitrite 2.09ppm, nitrate 21.21ppm, phosphate 0.21 ppm, ammonium 4.98 ppm, iron 2.94 ppm.

Electro kinetic reactor

The electrokinetic cells (Fig. 1) consisted of glass cells (inner dimensions: length 58cm, depth 15cm and width 15cm) divided into three compartments: two electrodes (10cm x 15cm x 15cm) with phosphate buffers (pH 7.8 in the anode and pH 5.8 in the cathode) using platinum electrodes inside the buffers, and a soil compartment (30cm x 15cm x 15cm) (Fig. 1). The experiments were run using a constant electric field of 0.5 V/cm. Moisture was monitored on a weekly basis with a gravimetric method, and maintained at about 12%.

Determination of hydrocarbons via Gas Chromatography analysis

Two grams of each individual sample was dissolved in 5 mL of pentane, phase separated, and percolated through 2 g of silica gel. One milliliter of elute was carefully evaporated until dry to determine the fuel oil content of the sample. The corresponding determination of semi-volatile compounds was made by GC–MS (gas chromatography AGILENT 7890 Plus, coupled to a mass spectrometry detector AGILENT), in accordance with EPA Method 8015 (HP GS/MS, equipped with a split/splitless injector, and a capillary column HP-5.) The injector and detector temperatures were maintained at 200°C 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 (275°C) was maintained for 5 minutes. Plate count of heterotrophic hydrocarbon-degrading bacteria.

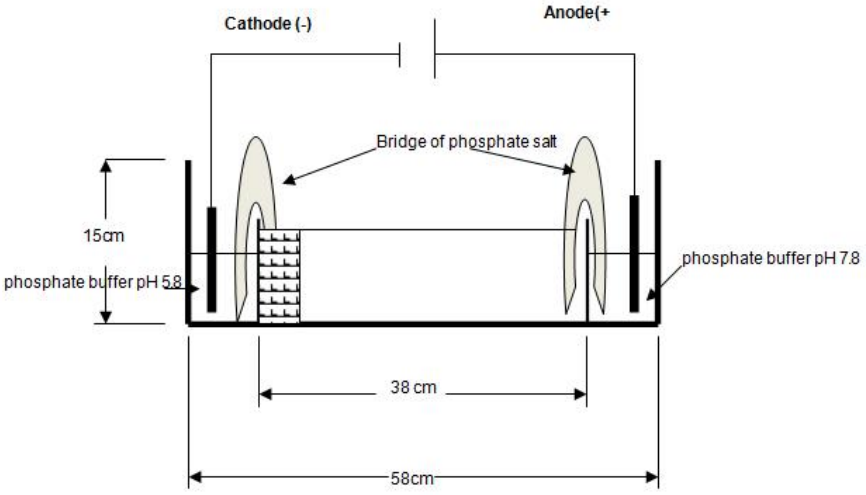

Figure 1. Design of the electrokineticreactor used to treat polluted sediment samples (Acuña *et al*., 2012)

Table 1. Nutrients, pH and bacterial count at the beginning of the experiment and at 69 days on bioremediation glasscells

	Initial	Cathode	Center	Anode	Control
Nitrate (ppm)	20.68	7.74	9.66	8.89	8.52
Nitrite (ppm)	2.101	0.53	0.74	0.45	0.56
Ammonium (ppm)	4.98	0.21	0.37	0.13	2.24
Iron (ppm)	2.91	4.27	6.97	3.91	4.98
Phosphate (ppm)	187	11.9	0.87	0.6	0.49
Sulfate (ppm)	329.2	247.02	349.05	330.77	274.04
pH	7.26	7.47	7.56	7.8	7.31
Conductivity (S/cm)	3793	2303	3522	3387	4741
R2A (CFU g^{-1})	1.63×10^5	1.05×10^5	8.78×10^9	8.38×10^4	1.35×10^5
MBM-PGO (CFU g^{-1})	5.71×10^7	1.56×10^8	9.9×10^7	2.1×10^8	1.32×10^9

In addition, a fertilized control without electric field was prepared. At the end of the experiment, samples were taken from the cathode, center and anodeof the solid cells for chemical analysis and enumeration of aerobic and degrading bacteria. Each sample was analyzed for nitrate (EPA 352.1), nitrite (SM 4500), pH (EPA 9040C), phosphate (SM 4500-C), ammonium (EPA 350.1), iron (ASTM 1068A), and sulfate (SM-4500E).

Several dilutions of the bacterial suspension were plated on R2A (Reasoner and Geldreich 1985)(yeast extract,0.5g; proteose peptone, 0.5 g; casamino acids, 0.5 g; glucose, 0.5 g; soluble starch, 0.5 g; K_2HPO_4, 0.3 g; $MgSO_4$ 7H2O, 0.05 g; sodium pyruvate, 0.3 g; agar, 15 g, suspended in distilled water), and incubated at 28°Cfor 28 days in the dark, and on MBM-PGO (Pucci and Pucci 2003) (NaCl 5 g, K_2PO_4H 0.5 g,

Table 2. Hydrocarbon concentration in the initial and final soil

Ppm	Initial	Control	Anode	Center	Cathode
< C8	0	0	0	0	0
C8 a C10	0	0	0	0	0
C10 a C12	0	0	0	0	0
C12 a C14	1994.62	1005.41	323.26	120.19	425.33
C14 a C16	7254.39	5329.01	2821.76	1981.15	3322.06
C16 a C18	11666.46	7931.85	4545.69	3183.63	5208.00
C18 a C20	14590.71	9733.34	5831.08	4182.47	6856.26
C20 a C22	14490.08	9876.26	5930.09	4282.57	7181.47
C22 a C24	9277.18	8161.93	5417.35	3983.37	7127.18
C24 a C26	8041.64	7700.35	5333.07	4165.92	7532.43
> C26	13426.31	13107.98	12915.48	10441.86	13001.86
Total	80741.4	62846.1	43117.8	32341.2	50654.6

Table 3. PAHs concentrations in the initial an d final soil

Ppm	Initial	Control	Anode	Center	Cathode
Naphthalene	127.004	98.959	3.897	3.283	3.496
Anthracene	220.942	138.334	19.263	13.545	16.912
Fluorene	25.544	14.982	0.284	0.194	0.283
Chrysene	23.595	16.694	4.807	3.508	5.274
Fluoranthene	17.143	11.215	5.232	3.814	5.738
Total	414.228	280.183	33.483	24.344	31.703

$NH_4PO_4H_2$ 0.5 g, $(NH_4)_2SO_4$ 1 g, Mg SO_4 0.2 g, KNO_3 3 g, $FeSO_4$ 0.05 g, suspended in 1L of distilled water). The surface of the agar plate was coated with 30 µL of a 1:1 mixture of petroleum-diesel oil and dried for 15 minutes at 65 °C and incubated for 28 days at 28 °C.

RESULTS AND DISCUSSION

The use of phosphate bridges allowed a better regulation of the pH levels in the soil (Fig. 1).It also allowed introducing nutrients to the soil,which led to an increase in the bioremediation of hydrocarbons (Table 1). The introduction of phosphate in the soil benefits biodegradation due to the fact that this nutrient is necessary where the concentration of nutrients is very low. Because of the electrical charge of the ions, migration occurred and it was modified nutrient the bioavailability of phosphates, a high concentration of phosphate is seen in the cathode, probably as a result of the bridges. In accordance with previous findings (Xuejun *et al*. 2006), the values of phosphate were modified (Table 1). In general, the surface of bacterial cells is negatively charged by electrophoresis (van Loosdrecht *et al*., 1989, Olszanowski *et al*., 2006) and electro-osmosis may move bacteria, depending on the soil (Mena *et al* 2012). However, in our results, the bacterial number was constant at 69 days because the cells were filled with unsaturated soil, 17%. Under electric field, the water and ions in the soil migrated toward the cathode, leading to conditions favorable for bacterial growth in the region adjacent to the cathode. However, the best hydrocarbon degradation occurred in the center. Moreover, the concentrations of nutrients such as phosphate ions were higher in the region near thecathode because of electro-osmosis and it given by saline bridge, nitrite, ammonium, Fe, decreased by microbial metabolism. Thus, the closer to the center the sample was, the richer its nutrients, resulting in a more favorable distribution of the degrading bacteria counts (Table 1), in concordance with that reported by Dong *et al*. (2013).

Minimizing pH changes at the electrodes can reduce stress responses in microorganisms (Lear *et al*., 2004). In the present study,the values of the bacterial counts experienced no modification. In all cases, the decrease of one logarithm was within the error of the method (Table 1). The bacteria did not migrate to the area of the electrodes, as stated by other authors in the case of saturated soil (Suni andRomantschuk, 2004).The number of hydrocarbon-degrading bacteria was enumerated using viable cell counting. The plate counts on TSA showed values below 10^8- 10^9 CFU/g,i.e. higher than those obtained in contaminated soils of Patagonia (Acuña *et al*., 2008, Pucci and Pucci 2003), and the grown were fast, in about 48 h. The bacteria develop hydrocarbon capacity as sole carbon and energy source showed slower growth and an important adaptation since they had more development. This high concentration of bacteria capable of degrading hydrocarbons indicates a significant potential for biodegradation in the samples (Atlas 1995,Bartha 1986), and this waste can be tretaed with the strains present, without having to develop bacterial inoculants, as reported by other authors (Van Hamme *et al*., 2003).This suggests that 0.5V/cm was not enough to kill bacteria and that the addition of inorganic nutrients was able to promote microbial growth in the soil. At the end of the electro-bioremediation, the number of hydrocarbon degraders was not significantly higher applied soil compared with the control. The bacterial counts in the soil appeared to have no modification, which is in agreement with the results of TPH removal. The TPH analysis showed differences between the values of hydrocarbons from the cells with 0.5 V/cm and those from the control cells. However, the values of the cathode, center and the anode were significantly different along the experiment (69 days). It was at this time that the nutrients were distributed in the cell (Table 1). The total aromatic hydrocarbons showed higher degradation in the center of the cell. The PAH contaminants were reduced throughout the cell, but degradation was greatest in the center, where the pH was most favorable for microbial activity.

Since PAHs are neutrally charged, electro-migration does not work for migration of these hydrocarbons across the soil specimens determined at the end of the experiments. Normally, crude oil tends to oxidation and biodegradation under natural conditions, even in the oil reservoir (Tables 2 and 3). Thus, crude oil always consists of a certain amount of oxygen-containing compounds, which can be attacked by monooxygenases. Moreover, some metabolites of petroleum hydrocarbons may persist in the soil during bioremediation of petroleum contamination (Table 2). The tank bottom sample consists primarily of hydrocarbon compounds. These can be classified according to their homologous series of linear hydrocarbons (16-28 carbon atoms) and branched hydrocarbons (15 to 30 carbon atoms), which have at their side chains and a methyl group derived from the allogeneic conjunction with polypropylenes. Most of these compounds are polysubstituted in more ring positions with simple methyl groups, which were degraded in the electrokinetic cells and in the control system. The methyl aromatic hydrocarbons and naphthalene were degraded in about 96.9% in the electrokinetic cells and in only 22% in the control system. The gas chromatography analysis of the sample in the cathode, center and anode indicated that oil was better degraded in the center (Tables 2 and 3). The hydrocarbons involved in the saturate fraction were identified in the range of C_{12} to C_{35} at the initial time of incubation. After 69 days of electro-bioremediation, the aliphatic fraction analyzed showed that the chromatographic profiles of this fraction had a different degradation pattern. The center of the electro-cell showed a preferable removal of C_{12} to C_{29} compounds. Hydrocarbon removal regularly occurs on a specific group of hydrocarbons. This specificity occurs due to the capacity of the microorganisms involved and according to their degrading enzyme system, as well as to the chemical nature of the hydrocarbons.

One of the problems of bioremediation is the bioavailability of hydrocarbons. The electrical field produced a hydrocarbon release of soil particles that may subsequently increase the bioavailability of hydrocarbons for the use by the microorganisms present in the soil (Pucci *et al*., 2012). Oil tank bottom samples have many hydrocarbons, which release compounds suchas naphthalene, anthracene, flourene, chrysene and fluoranthene. Different mechanisms were involved in the electro-bioremediation cell such as release of hydrocarbon from sediment particle, bacterial metabolic. According to Huang *et al*. (2012) if electro-bioremediation techniques continue to be developed and improved, and they willallow making important contributions to the remediation of contaminated soils when the soil cannot be remediated by land farming or biopiles. Thus, further studies on this issue should be carried out. The biodegradation process of oil tank bottoms is faster in aliphatic hydrocarbons such as n-alkanes of 13 to 26 carbon atoms, aromatic fractions that are somewhat slower to degrade and therefore require longer treatments to obtain satisfactory results. The application of low-intensity direct current for more effective remediation should improve the result obtained at the anode. According to Fan *et al*. (2015), biodegradation is stimulated by the electric field; it is a synergistic effect between electro-kinetics and biodegradation.

Electro-bioremediation is a promising technology for remediation of many organic contaminants in a problematic soil matrix when the alternative technologies may by ineffective (Gill *et al*., 2014). These results indicate that biodegradation and electro-osmosis can be successfully integrated to enhance PAH removal from oil tank bottoms and improve mobilization of the less bio-accessible fraction of PAH with an electro kinetic pretreatment to reach lower residual levels through bioremediation. This process may provide an effective technology for the treatment of problematic soil samples. 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.

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