Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 4, Issue, 12, pp. 451-454, December, 2012

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

THE USE OF ELECTROBIOREMEDIATION IN HYDROCARBON RELEASE AND BIOREMEDIATION

[1]Pucci Graciela, N., [1]Acuña Adrian, J., [2]Wick Lukas and [1]Pucci Oscar.H

[1]CEIMA -Universidad Nacional de la Patagonia San Juan Bosco, ruta pcial Nº1 4km, Comodoro Rivadavia-Argentina

[2]UFZ, Helmholtz Centre for Environmental Research, Department of Environmental Microbiology, Permoserstrasse 15, 04318 Leipzig, Germany **Corresponding author*: granapu@unpata.edu.ar

ARTICLE INFO

Article History:
Received 21th September, 2012
Received in revised form
18th October, 2012
Accepted 16th November, 2012
Published online 28th December, 2012

Key words:
Electrobioremedation,
Hydrocarbons release.

ABSTRACT

Hydrocarbon bioavailability is a relevant parameter for bioremediation technologies, especially in old contaminated sites. The aim of this work was to apply electrobioremediation (EKB) of sediments with two types of contaminant; in one case, it was an old hydrocarbon contaminant adsorbed by the sediment particle and in the second case, it was a laboratory added contaminant, which included phenanthrene and hexadecane. The removal of the added contaminant was fast, about 30 days in the case of the hexadecane and 61 days in the case of phenanthrene; but on day 91, naphtalin, 1- methylnaphtalin and 2- methyl naphthlin appeared and were then degraded except in the cathode zone. The use of EKB removed and bioremediated the old contaminant contained in the sediment sample.

INTRODUCTION

Over the years, the oil industry has been contaminating our region resulting in large surfaces of hydrocarbon contaminated soil. New contamination can be remediated by different techniques such as pump and treat methods, soil vapour extraction, bioventing, low temperature thermal desorption, dual phase extraction, chemical oxidations and bioremediation. However, there are many places with old oil-contaminated-soil, which is mainly composed by polyaromatic hydrocarbons (PAH) and high aliphatic hydrocarbon, >28C. These contaminants can persist in the environmental system for a long time. Most of them represent a risk to public health due to their carcinogenic or mutagenic properties, as benzopirene, and benzonthracene. In Patagonia, landfarming and biopiles are the most common bioremediation techniques applied to oil contaminated-soil, in spite of the fact that the Patagonian soil is low in nutrients, temperature, soil moisture, and there is low bioavailability of hydrocarbon because of particle adsorption, parameter that cannot be easily modified. One approach has been to combine bioremediation with electrokinetics (EK) into a hybrid technology, referred to as electro bioremediation (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. The EK has been proved in soil with different hydrocarbon contamination with good results, (e.g. PAH Niqui-Arroyo –Ortega Calvo 2010, Wick *et al* 2007, Shi *et al* 2008) The aim of the study was to test the effect of electro bioremediation on hydrocarbon removal from old contaminated hydrocarbon sediment with the addition of hexadecane and phenanthrene hydrocarbon.

MATERIAL AND METHODS

The sediment used in this study was taken from La Mata stream (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.

Electro kinetic reactor

The electrokinetic cells (EKCs) 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) (Fig. 1). The experiments were run using a constant electric field of 0.5 V/cm. Moisture was monitored on a weekly basis by a gravimeter method, and it was maintained at about 12%. In addition, fertilized control without electric field was prepared. EKCs were followed by nitrate (EPA 352.1), nitrite (SM 4500), pH (EPA 9040C), phosphate (SM 4500-C) and ammonium (EPA 350.1)

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 analysed and quantified by gas chromatography using a HP GS/MS, equipped with a split/splitless injector, and a capillary column HP-1 (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.

Enumeration of aerobic and degrading 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 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 on MBM-PGO (NaCl 5 g, K_2PO_4H 0.5 g, $NH_4PO_4H_2$ 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), 30 µL of a mixture 1:1 of petroleum-diesel oil was spread on the surface once set (Pucci & Pucci 2003) and plates incubated at 28 ºC for up to 21 days.

RESULTS AND DISCUSSION

Sediment samples contained a mixture of hydrocarbon due to the proximity of the stream to the productive areas of the San Jorge basin and the industrial area of the city. Part of the contamination was naturally bio remediated prior to the experiment, and only the hydrocarbon with a complex degradation, poor solubility, and low accessibility (because of the hydrocarbon being so absorbed by the sediment particles) was present. On the other hand, the concentration of hexadecane and phenanthrene was added. Both types of contamination, old and laboratory added contaminants acted differently due to the absorption of the contaminant by the sediment sample. The added hydrocarbons had a fast degradation depending on their solubility and toxicity in the cube with and without electric field. Hexadecane, because of its lineal structure and low toxicity, (Sikkema *et al* 1995) was rapidly degraded by Patagonian bacteria (Acuña *et al* 2012, Pucci *et al* 2000, Pucci *et al*., 2011). Hexadecane concentration decreased in about 30 days in the anode, centrum, cathode as well as in the fertilized control without electricity (Fig. 2). Phenanthrene was used, but its concentration lasted longer than the concentration of the hexadecane, the time period was about 60 days in the anode and centrum.

Table 1. Sediment chemical characteristic

			ppm		ppm
Moisture (%)	2.85	Pb	0.04	Cd	<0.13
Apparent Density (g cm^{3-1})	1.25	Cd	0.0005	Cr	<0.1
Real Density (g cm^{3-1})	1.77	As	0.1	Co	<0.23
Porosity %	29	Cl^-	921.51	Cu	<0.16
WHC (%)	30	NO_3^-	17.42	Fe	224.2
		SO_4^{-2}	413.19	K	37.7
%A	91.8	NH_4^+	0.4	Mg	99
%L	5.2	F^-		Mn	4.399
%a	3	Br^-	7.49	Na	889
Structure	Sand	PO_4^{-3}	2.63	Ni	<0.2
Conductivity (mS/cm)	121.8	CO_3^{-2}	218.65	P	<0.33
		pH	9.5	Pb	<0.2
		Al^{+3}	208.8	S	133.9
		Ba^{+2}	0.3863	Sr	0.3443
		Ca^{+2}	52.7	Zn	0.3536

WHC water holding capacity

Table 2. Effect of EKB on sediment content of hydrocarbon, migration of nutrients in the EK cell, and bacterial count on R2A and MBM-PGO media

	Initial	Anode	Centrum	Cathode	Control
Naphtalin	0.081	0.248	0.339	0.471	0.081
2-Methylnaphthalin	0.039	0.124	0.283	0.740	0.039
1-Methylnaphthalin	0.023	0.069	0.149	0.449	0.22
2.6 Dimethylnaphthalin	0.010	0.000	0.000	0.000	0.000
1.6 Dimethylnaphthalin	0.011	0.000	0.000	0.000	0.000
Acenaphthylen	0.001	0.000	0.000	0.000	0.001
Acenaphthen	0.003	0.000	0.000	0.000	0.003
Fluorene	0.097	0.060	0.058	0.220	0.094
Phenanthren	253.831	3.431	0.324	3.579	6.919
Pyren	0.000	0.014	0.020	0.098	0.017
n-Dodecan	0.075	0.000	0.000	0.000	0.0070
n-Tetradecane	0.248	0.000	0.000	0.000	0.000
n-Hexadecane	200.656	1.488	2.814	2.897	1.667
Pristane	0.000	0.000	0.000	0.058	0.000
n-Eicosane	0.000	0.000	0.000	0.045	0.000
Nitrate	101.7	14.5	14.7	15.5	97.3
Nitrite	3.16	0.17	0.25	0.17	0.77
Amonium	2.18	7.6	7.5	7.5	9.7
Phosphate	2	462.5	510	907.7	2.5
Rto R2A	1.74×10^{08}	3.50×10^{07}	8.50×10^{07}	2.86×10^{07}	6.70×10^{06}
Rto MBM-PGO	1.80×10^{07}	3.62×10^{07}	6.30×10^{07}	2.30×10^{08}	1.67×10^{08}
pH	8.4	7.1	7.9	9.6	8.3

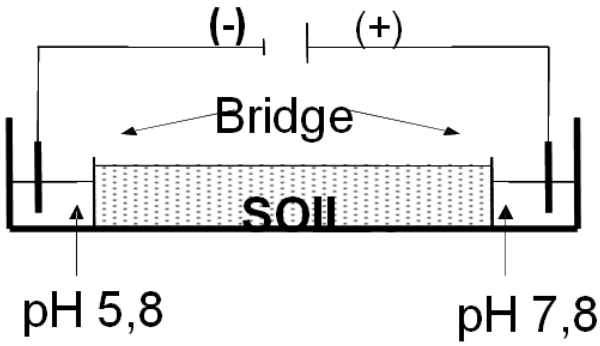

Figure 1. Design of the EK reactor used to treat polluted sediment samples

In the zone near the cathode, the penanthrene concentration increased during the first 91 days and then decreased (Fig 2). Despite the fact that the hydrocarbon has a neutral charge, it can be moved by means of electro osmosis to the cathode zone, in concordance with Park *et al*., (2005), but it is known that polyaromatic structure and its solubility (Ressler *et al*., 1999) are bioremediation problems. Phenanthrene concentration decreased slowly compared to what was found by Niqui Arroyo & Ortega Calvo (2010); due to this work, the field is an unsaturated soil, 12-15% of moisture.

Bioremediation in Patagonia soil is an effective technique (Pucci *et al* 2011, Acuña *et al* 2012), especially in warmer months (Pucci *et al*., 2011) and it is a very frequent technique. One of the problems of bioremediation is the bioavailability of hydrocarbon. The electrical field produced a hydrocarbon release of soil particle that these may subsequently increase the bioavailability of hydrocarbon for the use by the microorganisms present in the soil. The hydrocarbons: naphtalin, 1- methylnaphtalin and 2- methyl naphthalin increased their concentration and were then degraded (Fig 2). The same performance was in the fertilised control and the anode; on day 91, the concentration values increased and on day 154 the concentration values decreased. The centrum of the cube had the highest concentration values on day 120 and then these values decreased on day 154. The highest quantities of hydrocarbons realised was in the cathode, the principal was 2-methylnaphthalin, which was obtained in the end of the experiment, while 2.6-dimethylnaphthalin and 1.6 dimethylnaphthalin were degraded in the cube and in the fertilised control. Acenaphthylen, Acenaphthen, n-dodecan and n-tetradecane were degraded in the cube and did not degrade in the fertilised control. Some hydrocarbons as n-docosane fluoanathen, n-tetradeccane increased their concentration in the control, possible due to surfactants produced by

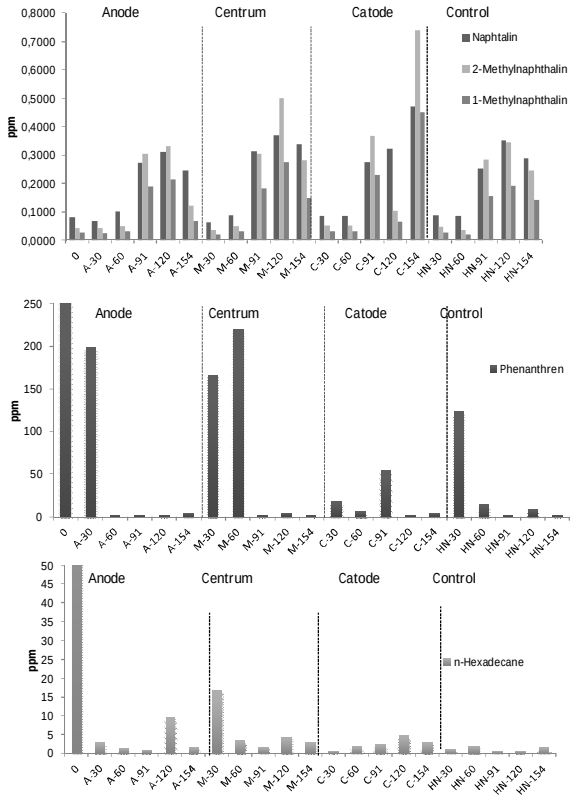

Fig. 2. Effect of EKB on Soil Content of Aromatic Compounds. A) naphthalin. 1.methylnaphthalin, 2metil naphthalin, b) hexadecane and c) phenanathreno

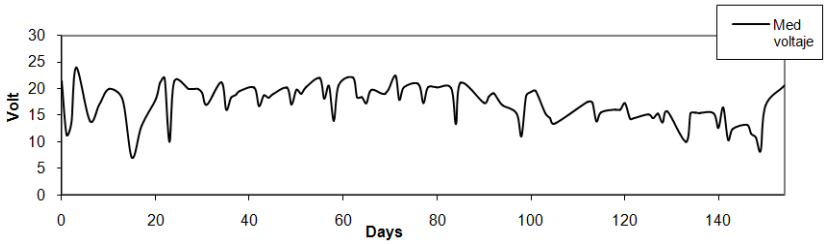

Fig. 3 Electric field on EKB cell during 154 days

microorganisms (Pérez Vargas *et al*., 2010). The pH changes in the cathode and anode zone are a problem for bacterial life. The introduction of a phosphate buffer maintained the pH in about 7- 8 (Table 2). Maintaining stable values was a problem in this type of unsaturated soil; the values fluctuated between 0.4V/cm and 1V/cm (Fig 3) because of the soil resistivity, which is normal in hydrocarbon contaminated soil. (Vazquez *et al* 2004). The phosaphate bridges provided with phosphate ions, necessary for the biodegradation process. The highest phosphate concentration was found in the cathode since its ion was provide by the bridges which is displaced to the area of the anode. The nitrate concentration decreased and the ammonium slightly increased values while there was the possibility of reactions:

$NO^3 + 2H^+ + 2e$ ---- $NO_2 + H_2O$ E0 ¼ þ0:84V
$NO^{2-} + 8H^+ + 6e$ -------$NH_4 + 2H_2O$ E0 ¼ þ0:89V
$2NO^{3-} + 12H^+ + 10\ e^-$ ---$N_2 + H_2O$ E0¼þ1:24V

The increment of nitrogen on the fertilised control would indicate the presence of amonificant bacteria (Deni and Penninckx. 1999). The hydrocarbon elimination had a good performance even in the fertilised control. However, in the cube with field, hydrocarbons appeared on day 91, which were later degraded. Counts values were normal in Patagonia soil and sediments, where the sample site has a history of contamination. Bacterial communities are adapted to low nutrient concentrations as nitrogen and phosphate salt, and can degrade hydrocarbon (Pucci *et al*., 2011). Economic cost involved in the implementation of electric field is only justifiable when the contaminated soil is very old and cannot be bioremediated by traditional techniques such as polyaromatic hydrocarbon, which can be removed and then degraded. There are many sites of old contaminated soil in San Jorge Basin, where the TPH values are never lower than the permitted legal values.

REFERENCES

Niqui-Arroyo J.L. and Ortega-Calvo JJ. 2010. Effect of electro kinetics on the Bio accessibility of Polycyclic Aromatic Hydrocarbons in Polluted Soils. *Reports:* Bioremediation and biodegradation, 39: 1993-1998.

Wick L.Y., Shi L. and Harms H.2007. Electro bioremediation of hydrophobic organic soil contaminants:a review of fundamental interactions. Electrochimica Acta, 52:3441-3448.

Shi L., Muller S., Harms H. and Wick L.Y. 2008. Effect of electrokinetic transport on the vulnerability of PAH-degrading bacteria in a model aquifer. Environ Geochem Health, 30: 177–18.

Reasoner D.J. and Geldreich E. E. 1985. A new medium for the enumeration and subculture of bacteria from potable water. Appl. Environ. Microbiol., 49: 1-7.

Pucci G.N. and Pucci O.H. 2003. Biodegradabilidad de componentes de mezclas naturales de hidrocarburo previamente sometidas a Landfarming. Revista Argentina de Microbiología, 35: 62-68.

Sikkema J., Bont J.A. and Poolman B. 2005. Mechanisms of membrane toxicity of hydrocarbons. Microbiology and Molecular Biology Reviews, 59: 201-222.

Acuña A.J., Pucci O.H. and Pucci G.N.2012. Effect of nitrogen deficiency in the biodegradation of aliphatic and aromatic hydrocarbons in patagonian contaminated soil. International Journal of Research and Reviews in Applied Sciences, 11: 479-485.

Pucci O.H., Bak M.A., Peressutti S.R., Klein I., Härtig C., Alvarez H.M. and Wünsche L.2000. Influence of crude oil contamination on the bacterial community of semiarid soils of Patagonia (Argentina). Acta Biotechnologica, 20: 129-146.

Pucci G.N., Acuña A.J. and Pucci O.H. 2011. Biodegradación de hidrocarburos en la meseta patagónica, un resumen de la optimización de los parámetros a tener en cuenta. Ingeniería Sanitaria y Ambiental, 115: 36–41.

Park J.Y., Kim S.J., Lee Y.J., Baek K. and Yang J.W. 2005. EK-Fenton process for removal of phenanthrene in a two-dimensional soil system. Engineering Geology, 77: 217-224.

Ressler B.P., Kneifel H. and Winter J. 1999. Biovailability polycyclic aromatic hydrocarbons and formation of humic acid-lke residues during bacterial PAH degradation. Applied and Micriobology Bacteriology, 53: 85-91.

Vázquez M.V., Hernández-Luis F., Grandoso D. and Arbelo C.D. 2004. Study of the Electrical Resistance of Andisols Subjected to Electro-Remediation Treatment. Portugaliae Electrochimica, 22: 399-410.

Deni J. and Penninckx M.J. 1999. Nitrification and autotrophic nitrifying bacteria in a hydrocarbon-polluted soil. Applied and environmental microbiology, 65: 4008-4013.

****