Polish J. Environ. Stud. Vol. 15, No. 2 (2006), 303-309

Original Research

The Use of an Electric Field to Enhance Bacterial Movement and Hydrocarbon Biodegradation in Soils

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Received: October 5, 2004
Accepted: August 31, 2005

Abstract

Bioremediation is the effective remediation technology for soils contaminated by biodegradable contamination. However, bioremediation of soils contaminated by hydrophobic compounds still remains a major challenge for the scientific and industrial world. There is still the need to develop techniques which allow an increase in bioremediation efficiency. A possible solution seems to be the stimulation of bacteria migration through the subsurface while using bioremediation.

In this study a weak electric field in combination with the following bacterial strains: *Pseudomonas putida*, *Bacillus subtilis* and *Klebsiella pneumoniae* was used to stimulate bacterial cell migration, as well as the biodegradation of crude oil contamination in soil samples.

Bacterial cell migration under the influence of the weak electric field and crude oil biodegradation were estimated during the experiments. The effect of changes in electrode polarization were also included in this study.

Results show that weak electric field application has a great influence on the speed and direction of bacterial migration in soil samples and biodegradation of the pollution. From the study of the application of the electric field in soil bacteria migration can be forced in the desired direction and consequently stimulate biodegradation of contamination in selected areas.

Keywords: bacterial migration, biodegradation electric field, electro-osmosis, electrophoresis

Introduction

In situ technologies are very popular methods of soil remediation. These technologies allow the recultivation process in a contaminated area without complex and expensive excavation work, which is mostly the case in *ex situ* technologies. Bioremediation, however, is affected by certain factors, which limit its efficiency. Some of the most significant factors are: presence of recalcitrant contamination in soils, very high level of soil contamination, and difficult geological conditions such as a low permeable clay presence limiting water and air migration in soil. Several methods of bioremediation enhancement have been developed to increase technological efficiency. The most effective have proven to be: both chemical and physical methods of soil aeration, nutrient application with mostly nitrogen and phosphorus compounds, additions of surfactants, additions of bacterial strains (bioaugmentation) and the electric field application.

The use of an electric field to force the transport of contaminants in the soil is called the electrokinetic method. This method of enhancement can be very effective under specific conditions [1-7] because it induces electro-osmosis, electrophoresis and electrolysis in the soil, which are strictly connected with contaminant migration in the subsurface.

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The transport process of contaminants consists of two phenomena:

1. solution flow according to Darcy's law and electrokinetic phenomena,
2. redistribution of dissolved compounds caused by diffusion and ion migration in the electric field.

Therefore, a resultant movement of dissolved contaminants in the electric field depends on the following factors: hydraulic fluid flow, electrokinetic flow of fluid, diffusion of dissolved compounds and migration of ions induced by electric field interaction.

Electrokinetic methods facilitate speeding up the transport and gathering of water-soluble contaminants in specially designed wells for their subsequent evacuation. Similar technology is also used to remove soil contamination caused by heavy metal ions, nitrates, and some polar organic compounds such as phenol or acetic acid [1, 2, 3, 5, 8-12].

However, this technological application is not successful for soils contaminated with hydrophobic compounds. The mobility of the contamination is greatly decreased because of the insolubility of hydrophobic compounds in water and their strong adhesion to soil particles. To date no successful technology has been found to purge non-polar organic substances from the soil. In order to overcome the poor contamination migration in soils, research on the behaviour of bacterial cells in the electric field was undertaken.

In terms of mobility, soil micro-organisms may be divided into two classifications: disperse micro-organisms (mobile or free swimming micro-organisms that have long tails or protozoa accelerating their movement through water) and aggregated micro-organisms (those which cluster and prefer an attached growth rather than remaining in a disperse phase) [13]. Most motile bacteria move by the use of flagella, rigid structures 20 nm in diameter and 15-20 μm, which protrude from the cell surface [14].

Due to the specific wall formation of the bacterial cell, numerous chemical groups are directed outside the cell, which results in a summary negative charge on the cell surface [15, 16]. Regarding the negative charge at the bacteria surface it is possible to speculate about bacterial movement under the influence of this electric field. Upon application of the electric field the pull of the negatively charged micro-organisms should be toward the anode and the one-dimensional flow of pore fluid from the anode to cathode [13]. However, bacterial behaviour in the electric field will strongly depend on field intensity. When the electric current density is 0.1-0.2mA/cm^2, the pH at the anode is stable in the range of 2-3 and pH at the cathode in the range of 8-12. These pH values are sufficient to destroy the acid-intolerant microbial species near the anode, while base-intolerant ones are killed at the cathode. Marks et al. (2000) found that the current density of 0.1-0.2mA/cm^2 is preferable in order to achieve a one-dimensional flow of pore fluid with bacterial strain from the anode to cathode [13]. Liu et al. [18] proved that electric field application stimulated directed bacterial movement. Bacterial movement under the influence of the electric field was studied in capillaries with an 8 μm inside diameter. The paper also demonstrated that the direction of bacterial movement strongly depends on electric field intensity.

The following work was carried out to estimate the influence of the weak electric field on the activity of selected bacterial strains in soils. Research also included studies of the impact of the electric field on the biodegradation of crude oil.

Materials and Methods

The experiments were carried out in two polyethylene tubes, (1 m length and 6cm width). The tubes were supplied with five bored holes, which were placed at the following points: cathode, anode, A, B, X_1, Y_1, X_2, Y_2, S_1, S_2, and enabled the sampling of soil from these points during experiments (Fig. 1). At the beginning of each experiment both tubes were filled with the contaminated soil, which was collected from a pipeline leakage area; the soil in the tube was not additionally compacted. The soil used in the experiments was clay soil with a hydraulic permeability coefficient below $1x10^{-7}$m/s. Average soil humidity was 13% (by weight). The average fuel oil hydrocarbon content in the soil was 3.11% (by weight). The hydrocarbon content was estimated based on the method of organic matter extraction from soil. Extraction was carried out at room temperature for 24h using diethyl ether as a solvent. Later, the solution was filtrated, dried, and the solvent evaporated and the remainder mass estimated.

The electrodes were placed at the ends of one tube and connected with the direct current source. The ends of the second tube were closed and it was used as a comparative system.

Suspensions of the selected bacterial strains were introduced to both systems at the central points (holes) S_1 and S_2. The average number of bacteria cells in the suspensions used for the experiments was $1*10^9$cells/ml. Most experiments were carried out in sterile conditions. In these cases soil, tubes and electrodes were sterilized before experiments.

During the experiment soil samples were collected from points (holes): A, B, X_1, Y_1, X_2, Y_2, S_1, S_2, cathode, anode to measure the number of bacterial strains. For that

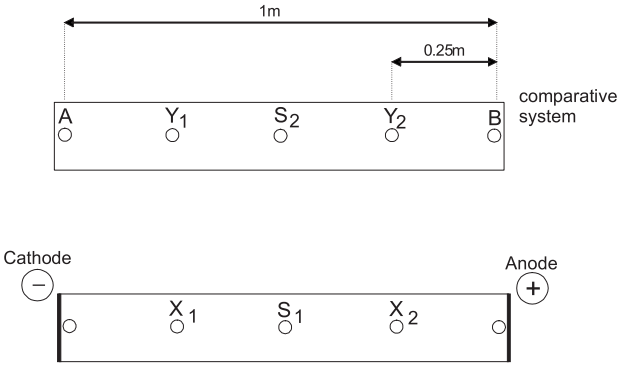

Fig. 1. Schema of the laboratory system.

Table 1. Results of the migration of *Pseudomonas putida* in the system under the influence of electric current and in a comparative system.

Time [day]	Number of bacterial cells of *Pseudomonas putida* [CFU/g] ($x10^{-7}$)									
	under the influence of electric field 10V/m					a comparative system				
	cathode (-)	X_1	S_1	X_2	anode (+)	A	Y_1	S_2	Y_2	B
7	0	0	4.8	0	0	0	0	6.4	0	0
14	0	0	1.8	0	0	0	0	6.8	0	0
21	0	0	6.0	9.6	0	0	0	1.5	0	0
28	0	0	4.0	5.0	0	0	0	6.0	0	0
35	0	1.0	0.9	1.2	0	0	0	1.4	0	0
42	0	0.01	2.0	1.0	0	0	0	3.0	0	0

Table 2. Results of the migration of *Klebsiella pneumoniae* in the system under the influence of electric current and in a comparative system.

Time [day]	Number of bacterial cells of *Klebsiella pneumoniae* [CFU/g] ($x10^{-7}$)									
	under the influence of electric field 10 V/m					a comparative system				
	cathode (-)	X_1	S_1	X_2	anode (+)	A	Y_1	S_2	Y_2	B
7	0	0	4.8	0	0	0	0	6.4	0	0
14	0	0	1.5	0	0	0	0	4.0	0	0
21	0	0	1.8	0.88	0	0	0	2.4	0	0
28	0	0	0.44	3.6	0	0	0	0.1	0	0
35	0	0	1.2	4.0	0.0001	0	0	2.0	0	0
42	0	0	1.0	0.28	0.24	0	0	1.3	0	0

purpose bacteria cells from the soil samples were extracted to the physiological solution and then seeded on an agar plate, incubated for 48 hours at 38˚C and counted. At the end of each experiment biodegradation degrees were evaluated at the same points using the above ether extraction method.

Pseudomonas putida (motile by means of one or more flagella), *Klebsiella pneumoniae* (nonmotile) and *Bacillus subtilis* (motile by means of one or more flagella) were used as separated bacterial strains. Also a biopreparation HBP-10 (GENESIS Technologies International, Buford, GA USA), containing *Pseudomonas*, *Bacillus*, and other bacteria was tested.

Results

The Influence of the Electric Field on the Migration of the Mixture of *Pseudomonas Putida* and *Klebsiella Pneumoniae* in Soil in Sterile Conditions

In this experiment mixtures of the bacteria *Pseudomonas putida* and *Klebsiella pneumoniae* were introduced to both systems. The experiment lasted 42 days, electric field intensity was 5V/m and current density at $6x10^{-3}$mA/cm^2. The pH at anode and cathode during the experiment were 7.0 ±1.0.

Both bacterial strains were observed at points X_2 after 21 days of experiments in the system, under the influence of an electric field (Table 1 and 2). Cells of *Klebsiella pneumoniae* were also detected at the anode point (+) after 35 days. These results indicate that bacterial migration is directed towards the anode under the impact of the electric field (electrophoresis). However, *Pseudomonas putida* bacterial cells were also detected at sampling point X_1 on day 35 of the experiment. This means that the electric field application also can stimulate bacterial movement toward the cathode. Bacterial migration in the opposite direction was probably caused by the electroosmotic migration of water through soil (electroosmosis). Instead, in the comparative system, bacterial migration was not observed as bacterial cells were detected only at a central point (S_2).

While bacterial migration to the anode was expected (negative surface charge of bacterial cells made them migrate towards the anode) bacteria migration to the cathode may be considered surprising. Bacterial migration in both directions (cathode and anode) may result from the competition be-

tween two phenomena: electrophoresis and electro-osmosis [15, 18, 19]. However, there may be other reasons than electro-osmosis that caused bacteria to migrate to the cathode. One of the reasons could be the migration of ions and water in soil toward the cathode under the influence of the electric field. This process could create favorable conditions for bacterial growth in the area near the cathode. This circumstance might cause bacterial cells to move to the cathode, although the net negative charge at their surface should make them move toward the anode. However, it is impossible to justify the most probable mechanism of the process based only on the achieved results of the experiments.

Very interesting results of contaminant biodegradation was observed after 42 days of the experiment (Fig. 2). Significantly advanced biodegradation of the contaminant was observed in the system under the influence of the electric field. The degree of biodegradation increases toward the anode and cathode similar to the increase of the number of bacterial cells (Fig. 2).

The Influence of the Electric Field and the Polarity of Electrodes on the Migration of the Bacteria Strain *Bacillus Subtilis* in the Soil in Non-Sterile Conditions

In this experiment the suspension of the *Bacillus subtilis* strain was introduced to both systems. The experiment lasted 91 days and on day 63 of the experiment the polarity of electrodes was changed, the electric field intensity being 5V/m and current density at $6x10^{-3}mA/cm^2$. pH at the anode and cathode during the experiment was 7.0 ±1.0. The obtained results (Fig. 3) indicate that the *Bacillus subtilis* bacteria also migrated to the anode (up to day 63 of the experiment). When the polarity of the electrodes was changed, the number of bacterial cells decreased at the "old" anode with an increase in the number of bacterial cells at the "old" cathode.

Influence of the Electric Field on the Migration of Micro-Organisms of the Hydrobiopreparation Group 10 (HBP-10) in Non-Sterile Conditions

The suspension of the biopreparation HBP-10 containing *Pseudomonas, Bacillus*, and other bacteria were introduced to both systems. The experiment was run for 56 days, maintaining the electric field at 5V/m and current density at $6x10^{-3}mA/cm^2$. The experiment was carried out in non-sterile conditions. pH at the anode and cathode during the experiment was 7.0 ±1.0.

The results obtained in the experiment (Tables 3, 4 and Fig. 4) show the influence of the electric field on the mixture of the *Pseudomonas* and *Bacillus* bacteria. It facilitates the migration of the microorganisms toward the anode, similar to a single strain of bacteria. In the system, under the influence of the electric field a larger biodegradation was observed than in the comparative system.

Conclusions

All experiments indicate the significant impact of the

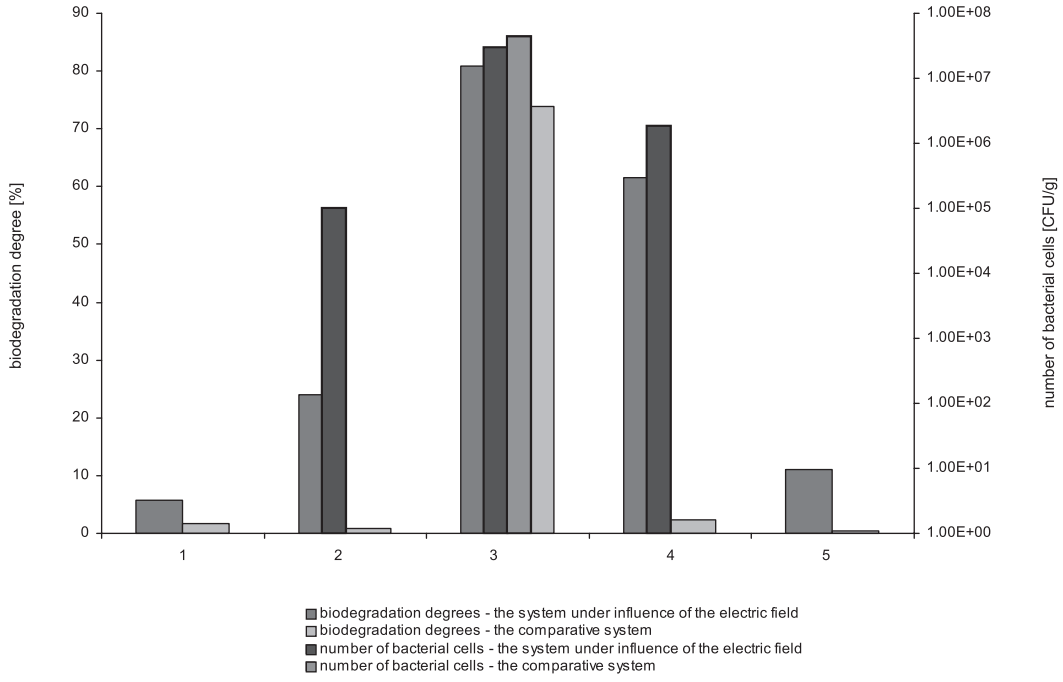

Fig. 2. Results of biodegradation of oil and a number of bacterial cells at the end of the experiment, in the system under the influence of the electric field and in a comparative system (42 days, *Klebsiella pneumoniae*, *Pseudomonas putida*), 1 – cathode, A; 2 – X_1, Y_1; 3 – S_1, S_2; 4 – X_2, Y_2; 5 – anode, B.

weak electric field on the activity of the selected bacterial strains: *Bacillus subtilis*, *Pseudomonas putida*, *Klebsiella pneumoniae* as well as the mixture of bacterial strains of *Bacillus* and *Pseudomonas* in the soil contaminated by crude oil.

The influence of the electric field with an intensity at 5V/m and current density at $6x10^{-3}mA/cm^2$ causes the migration of the bacterial strains toward the anode, as well as toward cathode (*Pseudomonas putida* cells were detected at points X_1 and X_2 in the system under the influence of the electric field, while in the comparative system the bacteria cells were present only at the central point). The bacteria movement toward the anode is induced by electrophoresis. However, it is difficult to judge the mechanism of the process of bacterial movement to cathode. Probably an electro-osmosis induces water migration in soil and electromi-

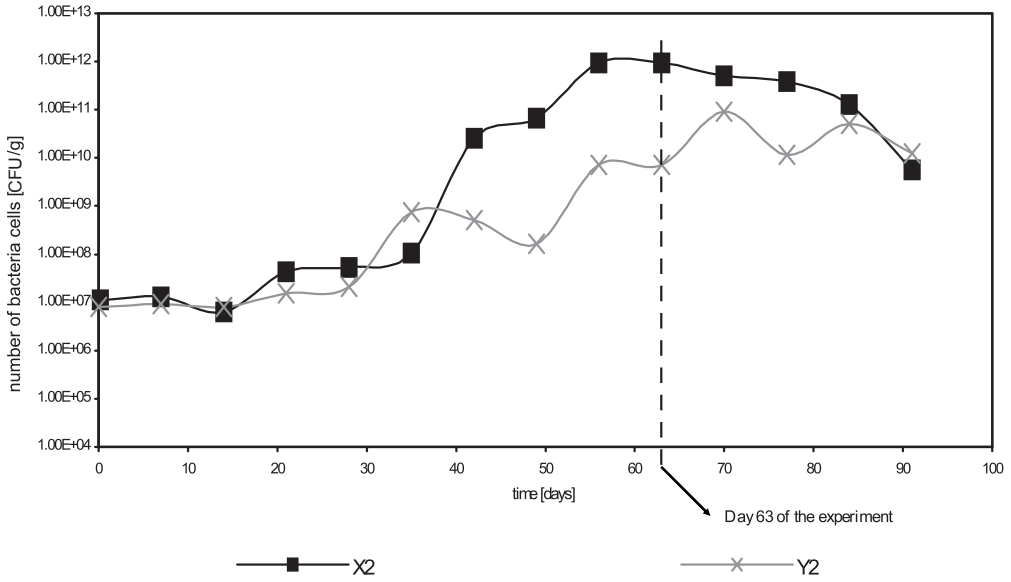

Fig. 3. Results of the migration of *Bacillus subtilis* bacteria in the system under the influence of the electric field (X_2) and in a comparative system (Y_2), (a change of a polarity of electrodes – day 63 of experiment).

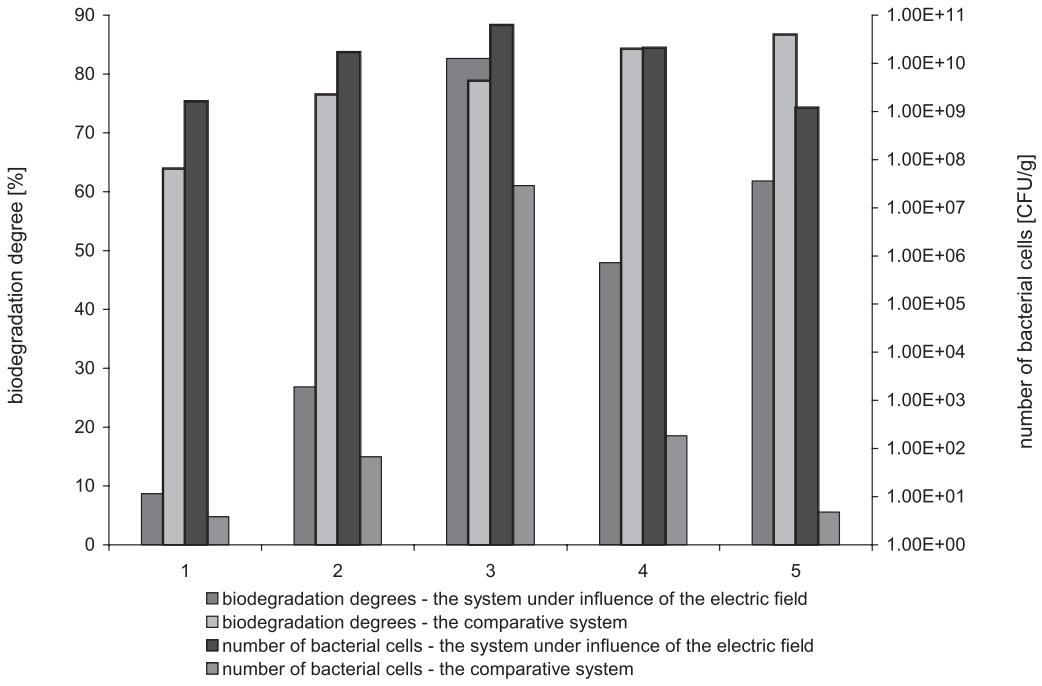

Fig. 4 Results of biodegradation of oil and a number of bacterial cells at the end of the experiment, in the system under the influence of the electric field and in the comparative system (56 days, the biopreparation HBP-10), 1 – cathode, A; 2 – X_1, Y_1; 3 – S_1, S_2; 4 – X_2, Y_2; 5 – anode, B.

Table 3. Results of the migration of a mixture of bacterial strains in the system under the influence of the electric current (the biopreparate HBP-10).

Time [day]	Number of HBP-10 bacterial cells [CFU/g]				
	anode	X_1	S_1	X_1	cathode
28	$4.37*10^8$	$5.06*10^8$	$2.68*10^8$	$1.46*10^8$	$9.41*10^8$
35	$1.58*10^{10}$	$9.10*10^9$	$4.47*10^8$	$2.57*10^8$	$7.48*10^7$
42	$2.00*10^{11}$	$2.00*10^{10}$	$1.39*10^{10}$	$2.30*10^8$	$2.30*10^8$
49	$1.35*10^{10}$	$1.83*10^{10}$	$9.08*10^9$	$3.67*10^9$	$7.47*10^7$
56	$3.86*10^{10}$	$1.97*10^{10}$	$4.27*10^9$	$2.20*10^9$	$6.41*10^7$

Table 4. Results of the migration of a mixture of bacterial strains in the comparative system (the biopreparate HBP-10).

Time [day]	Number of HBP-10 bacterial cells [CFU/g]				
	A	Y_2	S_2	Y_1	B
28	$7.48*10^7$	$4.57*10^8$	$1.45*10^8$	$9.76*10^8$	$5.36*10^7$
35	$5.44*10^6$	$2.83*10^7$	$1.53*10^{10}$	$8.60*10^6$	$9.22*10^6$
42	$2.10*10^8$	$1.90*10^8$	$5.72*10^{10}$	$9.85*10^8$	$2.60*10^8$
49	$2.71*10^9$	$1.87*10^{10}$	$4.54*10^{10}$	$1.45*10^{10}$	$9.96*10^9$
56	$1.59*10^9$	$2.06*10^{10}$	$6.12*10^{10}$	$1.67*10^{10}$	$1.18*10^9$

gration that stimulates ion migration. Both processes may create favorable conditions for bacterial growth. The results correspond well with previous studies on this topic, which indicate bacteria movement both in terms electrophoresis [15] and electroosmosis [18]. However, some research has to be done in this area to identify the mechanism.

It should be noticed that the electric field with an intensity at 5V/m and current density at $6x10^{-3}mA/cm^2$ favor bacterial movement toward the anode, which is opposite to the bacterial movement achieved during experiments with the greater electric current density (0.1 – $0.2mA/cm^2$ Marks et al [13] and 3.0 - $8.0mA/cm^2$ Wick et al [20,21]).

The change of the polarity of electrodes induces changes in direction of bacterial migration in the soil.

The weak electric field caused a large growth of bacterial cells at the sampling points compared to the soil without the electric field impact and resulted in greater oil biodegradation in the contaminated soil. For example, at the and of the experiment with HBP-10, at point X_1, the biodegradation degree was 12% greater than at point Y_1; and at point X_2 the biodegradation degree was almost 20% greater than at point Y_2.

References

1. ACAR Y. B., HAMED J. T. Removal of Cadmium (II) from Saturated Kaolinite by the Application of Electrical Current. Geotechnique **44** (2), 239, **1994**.
2. CHILINGAR G. V., LOO W. W. Electrobioremediation of Soils Contaminated with Hydrocarbons and Metals: Progress Report. Energy Sources **19**, 129, **1997**.
3. HICKS R. E., TONDORF S. Electrorestortation of Metal Contaminated Soils. Environ. Sci. and Technol. **28** (12), 2203, **1994**.
4. PROBSTEIN R. F., RENAUD P. C., SHAPIRO A. P. United States Patent. Electroosmosis Techniques for Removing Materials from Soil. Patent No. 5,074,986, **1994**.
5. YEUNG A. T., HSU C. EDTA – Enhanced Electrokinetic Extraction of Lead. J. Geotechni. Eng. 666, **1996**.
6. YEUNG A. T., DATLA S. Fundamental Formulation of Electrokinetic Extraction of Contaminants from Soil. Can. Geotech. J. **32**, 569, **1995**.
7. YIN J., FINNO R. J. Coefficient of Permeability from AC Electroosmosis Experiments. I: Theory. J. Geotech. Eng. 346, **1996**.
8. ACAR Y. B., GALE R. J. United States Patent. Electrochemical Decontamination of Soils or Slurries, Patent No. 5,137,608, **1992**.
9. HO S. V., BRODSKY P. H. United States Patent. In situ Remediation of Contaminated Heterogenous Soils, Patent No. 5,476,992, **1995**.
10. PROBSTEIN R. F., RENAUD P. C., SHAPIRO A. P. Preliminary Studies on the Removal of Chemical Species from Saturated Porous Media by Electroosmosis. PhysicoChemical Hydrodynamics **11** (5/6), 802, **1989**.
11. MARSHAL, K.C. Mechanisms of adhesion. in: Microbial Adhesion and Aggregation, Report on Dahlem Workshop on Microbial Adhesion and Aggregation. Berlin: Springer-Ver-

lag, 5, **1984**.
12. ELEKTOROWICZ M., BOEVA V. Electrokinetic Supply of Nutrients in Soil Bioremediation. Environ. Technol. **17**, 1339, **1996**.
13. MARKS R. E., ACAR Y. B., GALE R. J., OZSU-ACAR E. In-situ bioelectrokinetic remediation of contaminated soils containing hazardous mixed wastes in "Bioremediation of Contaminated Soils". Edited by Wise, D.L., and D.J. Trantolo, Cichon J. E., Inyang H. I., Stottmeister U., Marcel Dekker, Inc., New York-Basel, **2000**.
14. BERG H.C. Mobile behavior of bacteria. Physics Today. **53**, 24, **2000**.
15. BAYER E.M., J.L. SLOYER. The electrophoretic mobility of gram-negative and gram-positive bacteria: an electrokinetic analysis. J. Gen. Microbiol. **136**, 876, **2000**.
16. MESSNER P, SLEYTR U.B. Bacterial cell surface techniques. Wiley, Chichister, **1988**.
17. SHAPIRO A., PROBSTEIN R. Removal of Contaminants from Saturated Clay by Electroosmosis. Environ. Sci. Technol. **27** (2), 283, **1993**.
18. LIU Z., CHEN W., PAPADOPOULOS K. D. Electrokinetic Movement of *Escherichia coli* in Capillaries. Environ. Microbiol. **1** (1), 99, **1999**.
19. CHENG-CHUEH K., PAPADOPOULOS K.D. Electrokinetic Movement of Settled Spherical Particles in Fine Capillaries. Environ. Sci. Technol. **30**, 1176, **1996**.
20. WICK L.Y., MATTLE P.M., WATTIAU P., HARMS H. Electrokinetic transport of PAH-degrading bacteria in soils. Environ. Sci. Technol. **38**, 4596, **2004**.
21. WICK L.Y., MATTLE P.M., HARMS H., Electro-Bioremediation: The Impact of Direct Current on Transport and Activity of Pollutant Degrading Bacteria, Swiss. Med. Wkly. **133**, p. S6, **2003**.