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Full Length Research Paper

Bioremediation of diesel-contaminated soil using *Bacillus* sp. (strain TMY-2) in soil by uniform and non-uniform electro kinetic technology field

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The application of an direct current (DC) electric field (1 V/ cm) to diesel-contaminated soil specimens and the resulting electro kinetics phenomena has been combined with the *Bacillus sp*. strain TMY-2, to create a novel method for the remediation of soil contaminated with diesel fuel under uniform and nun-uniform electrokinetics. The effect of pH change was found to be particularly important in this respect, with acidic conditions hindering both movement and bioavailability. The transport and fate of this chemical were tracked throughout each experiment, along with properties of the soil pore fluid. Significant changes in soil chemistry were noted (particularly pH or moisture content). This method minimized the changes to these parameters within the soil. The result has shown the potential to mobilize and mix both bacteria and their substrates by electrokinetic transport processes mostly by the mechanism of electrophoresis and in part by electroosmosis. The electrokinetic distribution of strain TMY-2 through soil, under non-uniform electric field, was observed at ranging from 0.18-0.2 cm^2/ v/ h. An average diesel fuel removal was 71% (28 day) whereas, 41.4% diesel was removed on average in the same time period at the uniform electric field mode.

Key words: Bioremediation, electrokinetic, diesel fuel, *Bacillus cereus*, direct electric current.

INTRODUCTION

Petroleum hydrocarbon contaminants have been known to be present in many hazardous waste sites, which made an enormous impact on the quality of groundwater, soil and associated ecosystems (Kelsh, 1997; Groundwater Issue, EPA/540/4-91/002, 21 p, Estimating the Cold War Mortgage, 1995). Remediation of these contaminated sites to environmentally acceptable conditions is an important challenge facing the scientific and technical community because of heterogeneity of the soil matrix, of a sufficient density of bacteria or essential nutrients throughout the entire contaminated area, and the immobility of microbes or essential nutrient particularly when the soil pore space is partially filled with air (Acar et al., 1996; Bosma et al., 1997). Current *in situ* soil remediation technologies depend on hydraulic and air flow for effective remediation of soils and are not as effective in the clean-up of lower hydraulic conductivity soils (less than 10-5 cm/s) such as fine sands, silts and clays.

The presence of appropriate microorganisms at the actual site of contamination has long been recognized as a key factor in determining if biodegradation will occur, as well as in influencing the rate of biodegradation. A technology for uniform introduction of nutrients and microorganisms has been the principal bottleneck in the successful field implementation of in situ bioremediation (Zappi et al., 1993).

An emerging technology for treating petroleum contaminated-sites using in situ bioremediation methods is through the application of direct electric fields to enhance the contact probability of the bacteria and their hydrophobic organic compounds (HOC) by transporting bacteria to contaminant into heterogeneous and low permeability soils for the homogenization of microorganisms in soil (Wick and Harms, 2007) or inverse (Shi and Wick, 2008).

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Many laboratory (Lahlou et al., 2000), field-scale (Dybas et al., 2002; Major et al., 2002) and modeling studies (Schafer et al., 1998; Shein and Devin, 2002; Kim SB numerical analysis of bacterial, 2006) have been performed to understand and predict microbial transport in porous media.

As microbes are generally negatively charged, DC fields will cause their transport towards the anode (DeFlaun and Condee, 1997; Lee, 2001) and/or bacterial migration with the electroosmotic water flow to the cathode (Suni, 2004; Wick et al., 2004). These methods all rely on more selective and gentler ways to move microbes (and contaminants) through the soil and have in common that they do not require the excavation of soil or the mechanical mixing of the soil matrix. The success of using of electric fields depends on the specific conditions encountered in the field, including the types and amount of contaminant present, soil type, pH and organic content (Acar et al., 1996; Wick et al., 2004; Borroni and Rota, 2003).

When DC electric fields (approximately 0.2 – 2 V/ cm (Probstein, 1993) are applied to contaminated soil via electrodes placed into the ground, migration of charged ions occurs. Positive ions are attracted to the negatively charged cathode, and negative ions move to the positively charged anode. It has been experimentally proved that non-ionic species are transported along with the electroosmosis-induced water flow. The direction and quantity of contaminant movement is influenced by the contaminant concentration, soil type and structure, and the mobility of contaminant ions, as well as the interfacial chemistry and the conductivity of the soil pore water.

Recently, there has been an increasing interest in the bio-electrochemical processes in remediation of contaminated-fields. Studies showed that low level alternating currents (AC) and DC electric fields (in the range of volts/cm and up to few hundred Hz) stimulate the metabolic processes (Berg, 1993; McLeod et al., 1992; Blank et al., 1992) in a nonlinear way so that only a specific range of field strength and frequencies can cause a significant impact (Biochem, Biophys, Acta, 1113; Fologea et al., 1998).

Electric fields also introduce environmental changes that affect microbial growth and electrolysis reaction impacts pH, dissolved oxygen (DO) and other geochemical conditions. Furthermore, electric fields may produce an increase in temperature.

Several studies have demonstrated that microorganisms can be moved electrokinetically through soil, and even macro scale (up to 0.4 m), electrophoretic transport of bacteria and non-aqueous phase liquid (NAPL)-degrading yeast cells through sand, soil and aquifer sediments has been reported (Bodour, 1998; Jones et al., 1996; Van Schie, 1999; Pedersen, 1381). soil matrix, electrophoretic transport rates ranged from 0.019–0.023 cm^2/ h/ v for yeast cells (Van Schie, 1999) and 0.14–4 cm^2/ h/ v for bacteria (Bodour, 1998; Jones et al., 1996). This study explored the feasibility of using non-uniform electrokinetic transport processes to enhance distribution of new Bacillus strain TMY-2 Lab-scale non-uniform electrokinetic system with periodic polarity-reversal.

Here the influence of a weak DC-electric field typically used in electro-bioremediation (1 V/ cm) on the transport has been studied and distribution of the native diesel fuel-degrading bacteria and commercial diesel fuel as their growth substrate in a sandy clay soil under uniform and non-uniform mode were determined.

MATERIALS AND METHODS

The bacterial *TMY-2* used in this study was isolated from the top soil layer (30 cm in depth) petroleum hydrocarbon-contaminated soils around the oil refinery in Tehran (Iran), and performed in the media prepared from the following composition (g/ L): $NaNO_3$ (7); K_2HPO_4 (1); KH_2PO_4 (0.5); KCl (0.1); $MgSO_4$ _ $7H_2O$ (0.5); $CaCl_2$ (0.01); $FeSO_4$ _ $7H_2O$ 9. The medium was supplemented with 0.05 mL of trace elements solution of the following composition (g/ L): H_3BO_3 (0.25); $CuSO_4$ _$5H_2O$ (0.5); $MnSO_4$ _ H_2O (0.5); $MoNa_2O_4$ _ H_2O (0.06) and $ZnSO_4$ _ $7H_2O$ (0.7) (Mercade and Van, 1999).

The strain TMY-2 characterized and identified as diesel-degrading bacteria, based on colony morphology and pigmentation, and by performing Gram's staining and various biochemical tests prescribed in Bergey's manual of systematic bacteriology (Staley, 1989).

The sandy loam soil used in this study was collected from sites around the oil refinery in Tehran, Iran. After removal of surface litter, the soil was passed through a 2 mm sieve, sterilized three times by autoclaving and dried at 105°C. The organic content of dried soil was determined by the weight loss after a heating in a 550°C for 60 min. the average particle density of soil was calculated by dividing the known mass of soil by the volume change that was observed when the soil added to a column containing a known volume of water.

The diesel fuel oil, used for biodegradation experiment was a commercial grade fuel for truck that was purchased from a gasoline and diesel station in city of Tehran, Iran. The soil was contaminated with diesel to the concentration of 10,000 mg/kg and mixed homogeneously.

Amplification, cloning and sequencing of 16S rRNA gene

Genomic DNA of the isolate was extracted with a GenElute DNA extraction kit from Sigma by following the manufacturer's recommended procedure (Redburn and Patel, 1993). Amplification of genes encoding small subunit ribosomal RNA was carried out using Eubacterial 16S rDNA primers (forward primer 5' AGAGTTTGATCCTGGCT CAG 3' and reverse primer 5' ACGGCTACCTTGTTACGACTT 3' (Lane et al., 1985). PCR was carried out with the program initial denaturation at 95°C for 5 min followed by 10 cycles of 93°C for 1 min, 63°C for 1 min, 71°C for 1.5 min; 20 cycles of 93°C for 1 min, 67°C for 1 min, 71°C for 2 min and final extension at 71°C for 5 min. The purified PCR product was sequenced in both directions using an automated sequencer by SeqLab laboratory (Germany).

Diesel fuel extraction and analysis

Total petroleum hydrocarbon (TPH) was measured by infrared (IR) analyzer. The infracal modelcuvette holder (CVH) analyzer infracal TOG/TPH analyzer (Wilks Enterprise co.) is designed for use with EPA methods 413.2 and 418.1 (EPA publishes laboratory analytical

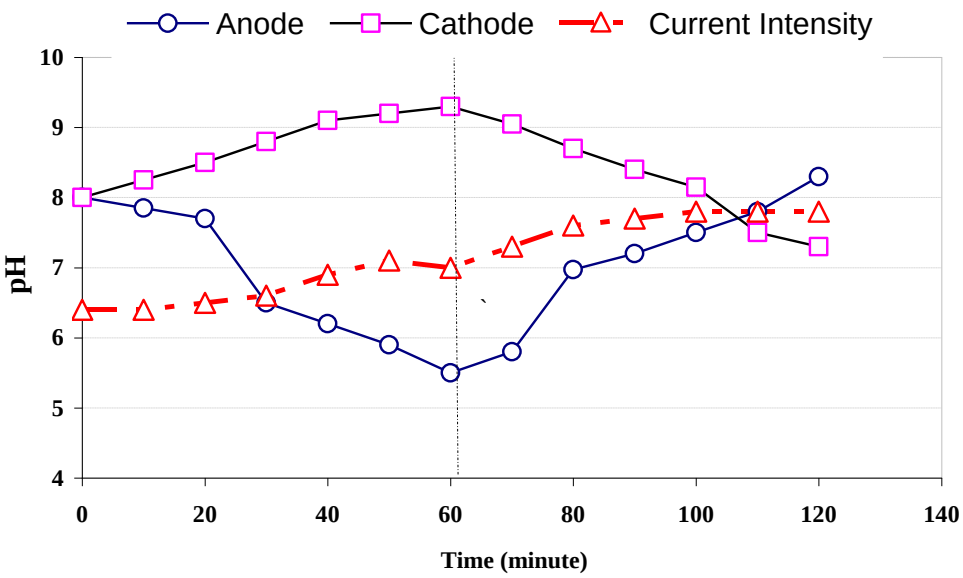

Figure 1. Electrolyte pH versus time.

methods that are used by industries and municipalities to analyze the chemical, physical and biological components of wastewater and other environmental samples that are required by regulations under the authority of the clean water act (CWA)). This is quick and easy field and laboratory analysis method for determining TPH, oil, and grease concentration levels in soil and water (EPA 530/SW-846, 1997). Perchloroethylene was used as an extraction solvent and purified extract was analyzed by infrared (IR) analyzer (Billets et al., 2001).

Experimental apparatus and cell transport analyses

The cell was constructed with plexiglas (length 20 cm × depth 6 cm × width 5 cm) body protected to prevent diesel fuel loss due to sorption and two electrodes were constructed from compressed graphite, with a regular array of holes drilled (approximately 2 mm diameter) to allow flow of electrolyte around them. Their dimensions were approximately 50 × 50 × 10 (mm), similar to that of the soil specimens, in order to ensure that all areas of the soil experienced a uniform electric field. Power was supplied by a bench-top power supply (maximum 40V). Filter papers were used at both ends of the specimen to avoid material loss and contamination of the electrode fluids by soil particles.

The contaminated soil was statically compacted at a pressure of 50 kPa in 100 g layers, with the final height matching the level in a rectangular top opened container and allowed to consolidate to make the bed of packing density 1.647 g/ cm^3 and of void fraction 0.49. A detail of the apparatus and experimental system is shown in Figure 1.

The separation distance between the electrodes was 15 cm. To promote maximum microbial and diesel fuel distribution in the soil matrix and controlled pH, these reservoirs were kept filled with a buffer solution with periodic polarity-reversal.

After packing, the soil was inoculated, along the line between the electrodes, with a cell density of 3 × 10^7 cells 1/ g, which was capable of using diesel fuel as a sole carbon and energy source for growth. One milliliter of a suspension was injected at a point that was half the depth of the bed equidistant from the center of the cell. The tests were treated in a room with a steady 25°C temperature for 14 days, during which the constant voltage applied was 1 V/ cm. To study the Electrokinetic distribution of *strain TMY-2* through the soil matrix, pore fluids in the soil were sampled at five points with distances of 1.5, 4.5, 7.5, 10.5, and 13.5 cm from the Anode chamber (0.1, 0.3, 0.5, 0.7, and 0.9 as a normalized distance from the anode). A control cell was maintained under exactly the same conditions (including saturation with water, inoculation, and the reservoirs were connected to 0.1 M phosphate buffer (K_2HPO_4/ KH_2PO_4) adjusted to pH 8.0 and the pH of either electrolyte could be controlled by manual addition of either sulphuric acid (at the cathode) or sodium hydroxide solution (at the anode). Another control cell was complicated without an electric field (for bioremediation test). However, an abiotic control was also run with soil autoclaved three times, which received no inoculums. After treatment, soil was analyzed.

The electrode control apparatus was capable of reversing the polarity of electric field, thus allowing changing the operation mode during the tests. In the system electric current and voltage could be monitored on-line and stores them into a personal computer for later analysis. The power supply could provide a constant DC electric voltage in a range from 0 to 40V for the electrokinetic tests.

Four separate groups of tests were conducted. The first was to investigate the mobilization of commercial diesel fuel and the change of soil pH and moisture when adopting different operation mode (uniform and nun uniform electric field). The second was to evaluate the bioremediation of diesel coupled with whole inoculation. The third and fourth were to evaluate the biodegradation of phenol coupled with uniform and non-uniform electrokinetic under local and whole inoculation, respectively. Control tests, with no electric field applied or no bacteria inoculated, were run in parallel.

Statistical analyses on experimental data were performed using SPSS Standard Version software (release 11.5.1 - SPSS Inc., Chicago, USA). University and multivariate analyses of data from individual experiments were performed using analysis of variance (ANOVA), in order to compare several means of three replicate samples at once. Results are presented with error bars indicating 95% confidence intervals, unless indicated otherwise.

Table 1. Comparition of biochemical and physiological characteristics of TMY-2 with *B. cereus* and *B. thuringiensis.*

Feature	TMY-2 Strain	*B .cereus*	*B. thuringiensis*
Cell morphology	Rod	Rod	Rod
Spore	+	+	+
Gram reaction	+	+	+
Catalase	+	+	+
Oxidase	+	–	+/-[(b)]
Motility	+	+/-[(b)]	+
pH range for growth	6-11	5.6-6.8	5.6-6.8
Acid from:			
D-Mannose	–	–	–
D-Fructose	+	+	+
Maltose	+	+	–
Arabinose	+	–	–
D-Glucose	+	+	+
Lactose	–	–	–
Galactose	–	+/-[(b)]	+/-[(b)]
D-mannitol	+	–	–
Sucrose	–	+	–
D-Salicin	+	+	–
Hydrolysis of:			
Starch	–	+	+
Tween 80, DNAse	–	+/-[(b)]	+
DNAse	+	+/-[(b)]	+
Gelatin	+	+	+
Urease	+	+/-[(b)]	+/-[(b)]
Indole production	–	–	–
MR	–	–	–
VP	+	+	+/-[(b)]
Utilization:			
Inulin	–	–	–
D-fructose	–	+	+
D-galactose	–	–	–
Sucrose	–	–	–
H_2S production	–	……	……
Nitrate reduction	–	……	……
Lactose	–	–	–

a +, 90-100% of strains are positive, c 11-89% of strains are positive.

RESULTS AND DISCUSSION

The strains TMY-2 were identified as *B. cereus and B. thuriengiensis* respectively and were verified by phenotypic analysis. Bothe the strains were found to be positive for catalase and oxidase tests. The *TMY-2* was positive for Urease and the Voges–Proskauer reactions, and did not hydrolyze starch and Tween 80. The details of other morphological and physiological characteristics are summarized in Table 1.

Species of the genus *Bacillus* are rods, which sporulates in aerobic conditions. The endospores are resistant to heat, dehydration, or other physical and chemical stresses.

A typical pH plot versus time is shown in Figure 1; showing the catholyte pH rapidly increased to approximately pH 9.2, whereas the anolyte pH rapidly decreased to approximately pH 5.5. These changes occurred in approximately 1 hour, and this behavior was seen in all experiments without pH control. Reversing the current every 1 h would allow pH changes to be naturally controlled, as hydrogen ions produced in 1 h period would largely be neutralized by hydroxyl ions produced in the next hour.

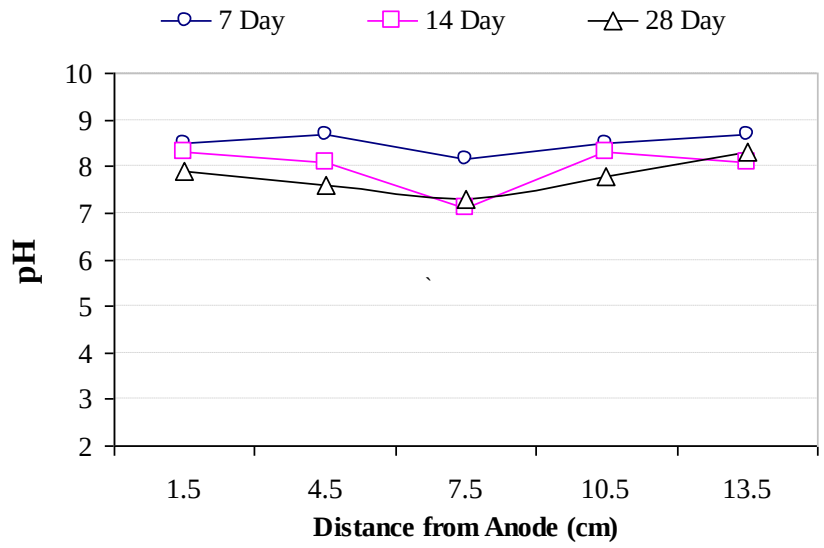

Figure 2. Variation of pH profile with time.

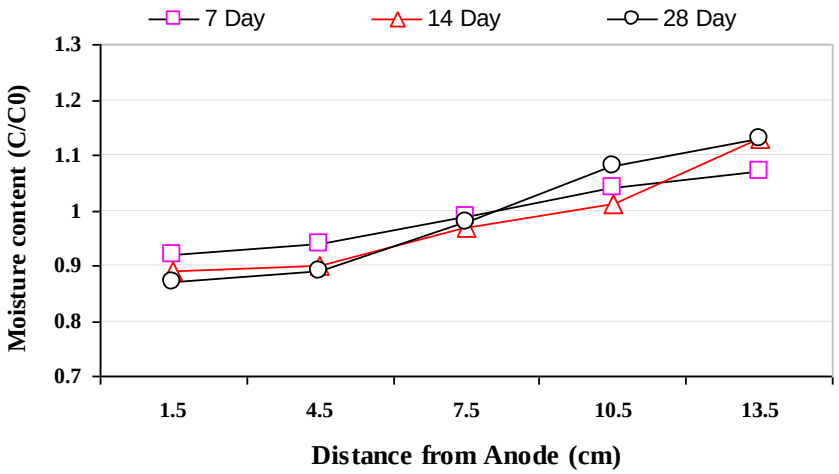

Figure 3. Moisture content distribution uniform electric field.

However, the pH of soil pore fluid was measured at every sampling location. In the experiment, once the constant voltage applied, the acidic and alkaline pH fronts moved through the soil, with the average pH at the anodes and cathode decreasing and increasing by up to 5 and 4 pH units, respectively by the end of testing (data not shown). The results of pH values with time is shown in Figure 2. demonstrating a regular reversal of current controlling pH in the soil (as there was no electrolyte fluid and therefore, pH could not be controlled by any other means). This can be seen to have worked, as there are only very small drops in pH at either end due to an electric current, after 7, 14, and 28 days of testing.

The moisture content of soil during experiment was determined for each soil sample taken, in order to calculate contamination levels per dry weight of soil. Some small errors in its calculation may have been introduced due to the effect of compaction during sampling, as the soil corer was pushed in. These tests were implicated to prevent the saturation of the soil. This was done by removing the need for electrolyte fluids – electrodes were placed in direct contact with the soil.

The applied DC field induced the change in soil moisture depending on the operation mode shown in Figures 3 and 4.

After running in uniform operation, the soil moisture near the anode was dropped by 6 to 11%, while near the cathode increased by 8 to 12% (Figure 3). The extreme soil consolidation due to water loss was considered not favorable for the biodegradation of

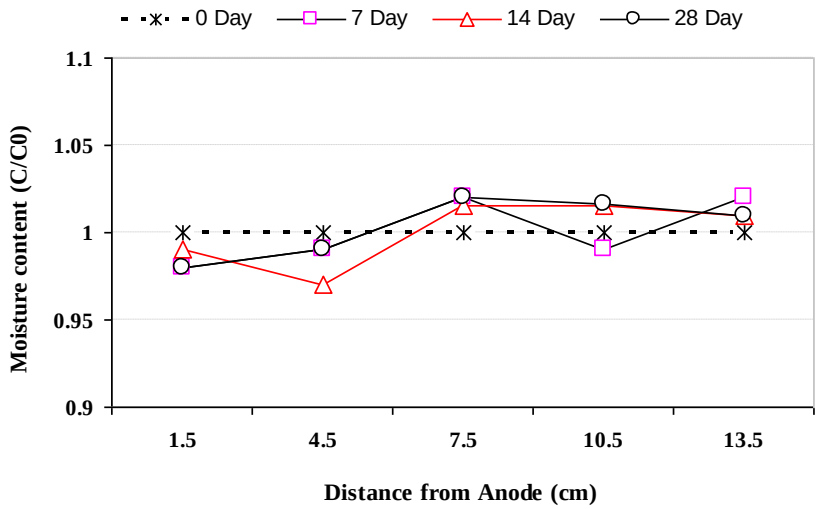

Figure 4. Moisture content distribution in non–uniform electric field.

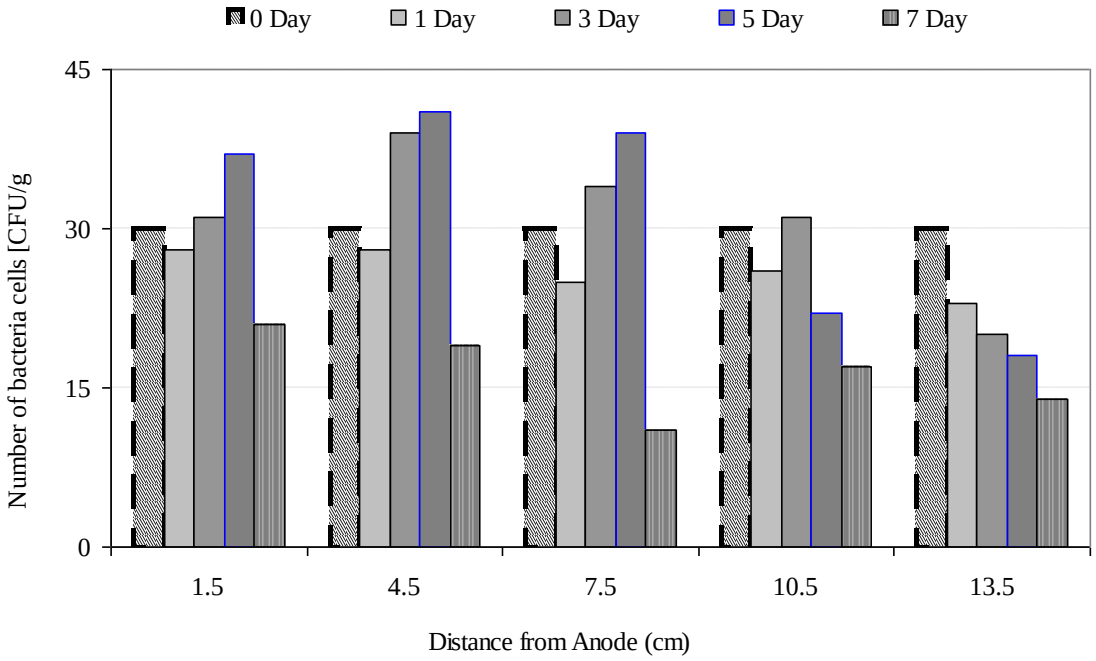

Figure 5. The distribution of TMY-2 strain (shown in blue color) by uniform electric current.

organic pollutants in soils. As contrast, when reversing the polarity of electric field at intervals of 1 h, non – uniform condition, and the soil moisture changed no more than 5% (Figure 4), indicating that the soil properties could be kept undisturbed by reversing the polarity of the applied electric field. Therefore, two directional operations may be favorable for in situ bioremediation. The applied DC field induced short-term experiments were conducted to analyze the transport of bacteria in contaminated soil influenced by direct electric current.

Diesel-degrading cell (TMY-2 strain) was injected into the whole specimen of packing soil and exposed to electric fields of 1 V/ cm with I = 0.5 mA/ cm^2 for 7 day. Total numbers of bacteria were quantified at various locations. DC electric current uniform or non-uniform caused that most of the sampled cells had been transported by electrophoresis (Figures 5 and 6). However as shown in Figure 6, non-uniform electric current enhanced the number and the distribution of bacteria cells in time and space. The similar finding was reported by Lee et al.,

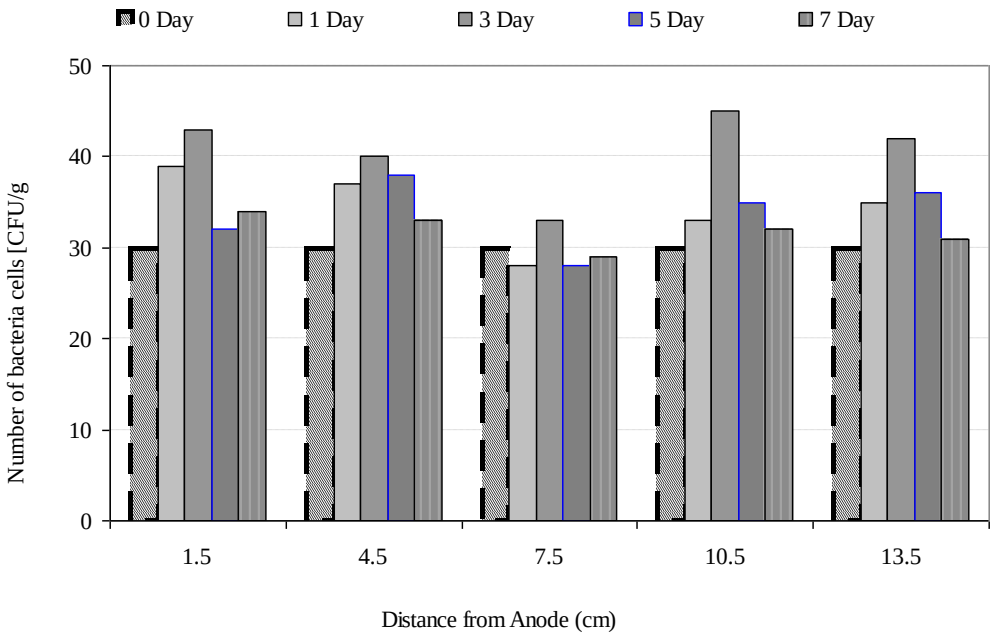

Figure 6. The distribution of TMY-2 strain (in blue color) for non uniform currents.

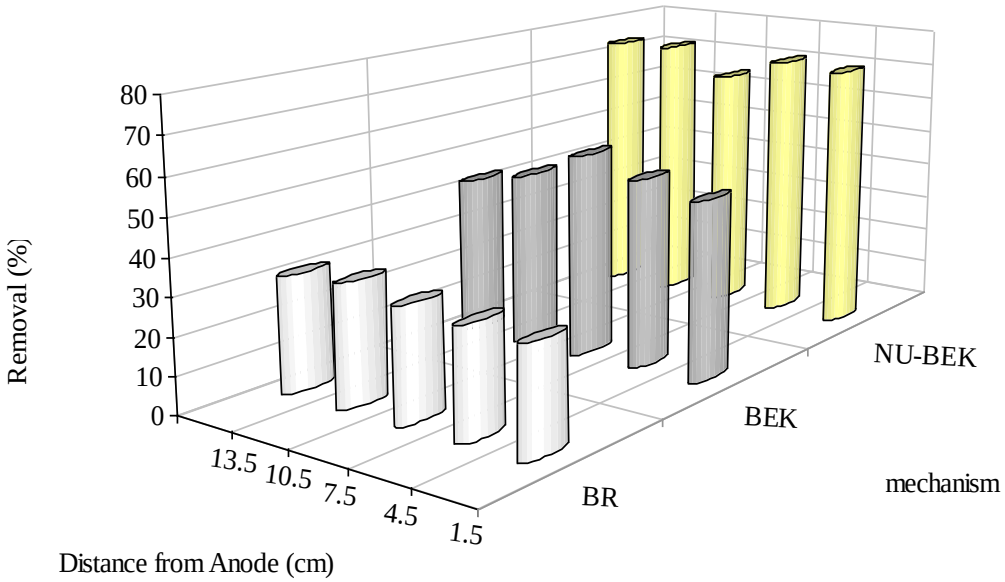

Figure 7. The efficiency of removal of diesel with uniform and non uniform DC power.

(1999). Figures 5 and 6 Shows the number of bacterial cells reached in maximum in the middle of cell for 5 days and then reduced.

In order to find out the most important factor which affect the removal the surface tension between the particles were determined and demonstrated in Figure 7. As shown in the case of non-uniform almost surface tension reduced in half (Figure 8).

Conclusions

In this research the application of direct current (DC) electric field (1 V cm^{-1}) on contaminated-soil injected with Bacillus sp. strain TMY-2, a hybrid technology of bioremediation and electrokinetic, for the treatment of soil contaminated with hydrophobic organic compounds (diesel fuel) under uniform and non-uniform DC-

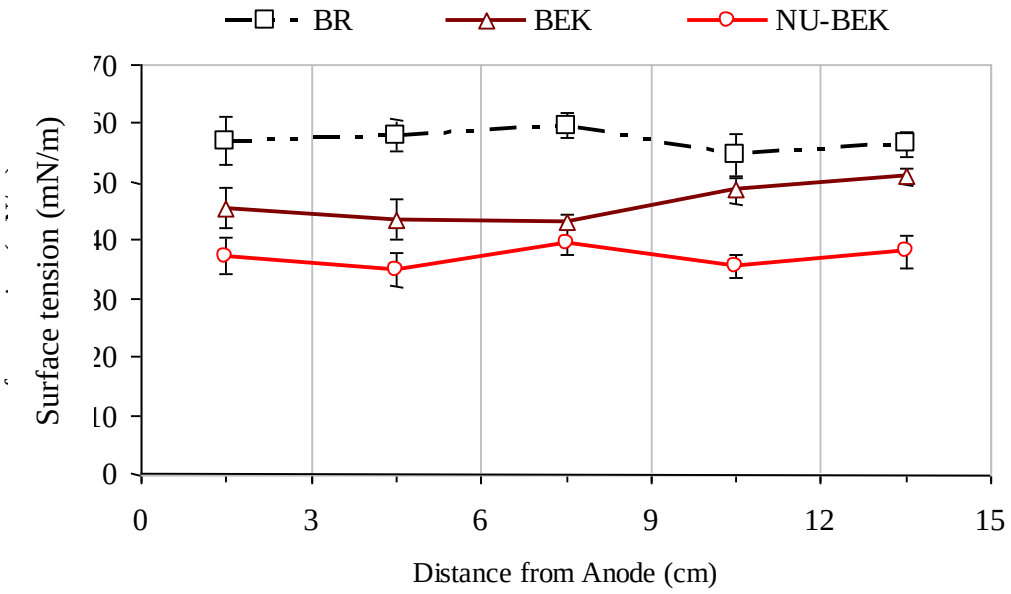

Figure 8. Surface tension results for uniform and non- uniform currents.

electrokinetic was investigated. Non-uniform electrokinetic processes can effectively enhance the desorption and movement of diesel in sandy loam. Under unidirectional operation, the diesel was moved and accumulated to specific regions. Non-uniform electrokinetics can accelerate effectively the biodegradation of diesel in sandy loam, the efficiency of which is related to the operation mode. Reversing the polarity of electric field could effectively enhance the biodegradation of diesel in the soil depending upon the polarity-reversing interval. Unidirectional operation could also accelerate the diesel biodegradation, but it caused higher diesel remains in specific regions. Unidirectional operation induced the extreme soil pH and soil consolidation, whereas bidirectional operation could maintain the soil pH and moisture at large if adopting a proper polarity reversing interval. However, polarity-reversal increased the electricity consumption; the faster the polarity reversal, the greater the electricity consumption. It has the potential to mobilize and mix both bacteria and their substrates by electrokinetic transport processes mostly by the mechanism of electrophoresis and in part by electroosmosis depending on their physico-chemical surface properties, because the cells acted as negatively charged particles at neutral pH. Current study showed weak DC-electric field able to mobilize diesel fuel-degrading bacteria at the millimeter scale and hence to increase the local diesel fuel-bioavailability in soil medium and the bioremediation efficiency. The overall electrokinetic distribution of strain TMY-2 through sandy loam soil, under uniform and non-uniform electric field, was observed at ranging from 0.125 to 0.150 and 0.18-0.2 cm^2/ v/ h respectively. At the non-uniform mode, an average diesel fuel removal of 66.5% was achieved in 14 days, whereas, 41.4% diesel wasremoved on average in the same time period at the uniform electric field mode.

REFERENCES

Acar YB, Rabbi MF, Ozsu EE, Gale RJ, Alshawabkeh AN (1996). Enhance soil bioremediation with electric fields. Chemtech., pp. 40-44.

Berg H (1993). Electrostimulation in Cell Biology by Low Frequency Electromagnetic Fields," Electro Magnetbiol., 12: 147-163.

Billets SN, Topudurti K, Tay S (2001). Innovation technology verification report "Field measurement technology for Total Petroleum Hydrocarbons in soil" Wilks enterprise, inc. Infracal TOG/TPH analyser.U.S.Environmental protection agency, washington, DC, EPA/600/R-01/088 (NTIS PB2002-101361).

Blank M, Soo L, Henderson AS, Goodmann R (1992). "Changes in Transcription in HL-60 Cells Following Exposure to Alternating Currents from Electric Fields," Bioelectrochemistry and Bioenergetics, 28: 301-309.

Bodour AA (1998). RMM-Application of a modified drop-collapse technique for surfactant quantitation and screening of biosurfactant-producing microorganisms, J. Microbiol. Methods, 32: 273-280.

Borroni A, Rota R (2003). Combined slurry and solid-phase bioremediation of diesel contaminated soils. J. Hazardous Materials, 4041: 1-16.

Bosma TNP, PJMM, G Schraa, AJB Zehnder (1997). Mass transfer limitation of biotransformation: quantifying bioavailability, Environ. Sci. Technol., 31: 248-252.

DeFlaun MF, Condee CW (1997). Electrokinetic transport of bacteria. J. Hazardous Materials, 55(1): 263-277.

Dybas MJ, Heine R, Tiedje J, Linning K, Wiggert D, Voice T, Zhao X, Dybas L, Criddle CS (2002) Development, operation, and long-term performance of a full-scale biocurtain utilizing bioaugmentation. Environ. Sci. Technol., 36: 3635-3644.

EPA (1997). Methods for evaluating solid waste: Physical/ chemical methods, Environmental Protection Agency,. Publication No. EPA 530/SW-846.

Fologea D, Vassu-Dimov T, Stoica I, Csutak O, Radu M (1998). "Increase of Saccharomyces Cerevisiae Plating Efficiency After Treatment with Bipolar Electric Pulses," Short Communication, Bioelectrochem. Bioenergetics, 46: 285-287.

Jones DS, CGA, Mawhinney WM, SP Gorman (1996). Standardisation and comparison of methods employed for microbial cell surface hydrophobicity and charge determination, Int. J. Pharm., 131: 83-89.

Kelsh DJaP (1997). "Department of energy sites suitable for electrokinetic remediation" Journal of Hazardous Materials, Special Edition on Electrochemical and V. Decontamination of Soil and Water, pp. 1-3.

Kim SB (2006). Numerical analysis of bacterial transport in saturated porous media. 2006. 20.

Lahlou M, Springael D, Ortega-Calvo JJ (2000). Influence of soil components on the transport of polycyclic aromatic hydrocarbon degrading bacteria through saturated porous media and EST, 34: 3649-3656.

Lane DJ, Pace B, Olsen GJ, Stahl DA, Sogin ML, Pace NR (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc. Nat. Acad. Sci. USA, 82: 6955–6959.

Major DW, Cox EE, Edwards EA, Dworatzek SM, Hendrickson ER, Starr MG, Payne JA, Buonamici LW (2002). Field demonstration of successful bioaugmentation to achieve dechlorination of tetrachloroethene to ethene. Environ. Sci. Technol., 36: 5106-5116.

McLeod BR, Liboff AR, Smith SD (1992). "Biological Systems in Transition: Sensitivity to Extremely Low Frequency Field, Electro Magnetbiol., 11: 29-42.

Mercade PM, Van Schie MF (1999). Adhesion of biodegradative anaerobic bacteria to solid surfaces, Appl. Environ. Microbiol., 65: 5082-5088.

Pedersen K (1381) A method for assaying the relative surface charges of bacteria, FEMS Microbiol. Lett., 12: 365-367.

Redburn AC, Patel BKC (1993). Phylogenetic analysis of Desulfotomaculum thermobenzoicum using polymerase chain reaction-amplified 16S rRNA-specific DNA. FEMS Microbiol. Lett., 13: 81-86.

Schafer A, Harms H, Stauffer F, Dracos T, Zehnder AJB (1998) Transport of bacteria in unsaturated porous media. J. Contam. Hydrol., 33: 149-169.

Shein EV, Devin BA (2002) Transport of microorganisms in soils: Physicochemical approach and mathematical modelling. Eurasian Soil Sci., 35: 500-508.

Shi L, Wick LY (2008). Electroosmotic flow stimulates the release of alginate-bound phenanthrene. Environ. Sci. Technol., 42: 2105-2110.

Suflita JMaS GW (1991). "Anaerobic Biotransformation of Contaminants in the Subsurface," EPA, Robert S. Kerr Environmental Laboratory, Ada, OK, EPA/600/M- 0/024.

Suni SRM (2004). Mobilisation of bacteria in soils by electro-osmosis. FEMS Microbiol. Ecol., 49: 51-57.

Wick LY, MP, Wattiau P, Harms H (2004) Electrokinetic transport of PAH-degrading bacteria in model aquifers and soil. Environ. Sci. Technol., 38: 4596-4602.

Wick LY, Harms H (2007) Electro-bioremediation of hydrophobic organic soil-contaminants: A review of fundamental interactions. Electrochim. Acta, 52: 3441-3448.

Zappi M, Gunnison D, Pennington J, Cullinane J, Teeter CL, Brannon JM, Myers T (1993). "Technical Approaches for *In situ* Biological Treatment Research: Bench- cale Experiments," US Army Corps of Engineers, Waterways Experiment Station, Vicksburg, MS, August 1993, Technical Report No. IRP-93-3.