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Bioremediation of Diesel-Contaminated Soil by Bacterial Cells Transported by Electrokinetics

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Abstract The electrokinetic technology was applied in bioremediation for the purpose of supplying a *Pseudomonas* strain capable of degrading diesel to contaminated soil bed, and their biodegradation of diesel was carried out after a desired cell distribution was obtained. Electrokinetic injection of the strain was made possible because the cells acted as negatively charged particles at neutral pH, and thus the cells were transported with a precise directionality through the soil mostly by the mechanism of electrophoresis and in part by electroosmosis. A severe pH change in the soil bed was formed due to the penetration of electrolysis products, which was harmful to the cell viability and cell transport. To achieve a desirable cell transport and distribution, the control of pH in soil bed by a recirculating buffer solution in electrode chambers was essential during the application of an electric field. The judicious selections of electrolyte concentration and conductivity were also important for achieving an efficient electrokinetic cell transport since a higher electrolyte concentration favored the maintenance of pH stability in soil bed, but lowered electrophoretic mobility on the other hand. With electrolyte solution of pH 7 phosphate buffer, a 0.05 M concentration showed a better cell transport than 0.02 M and 0.08 M. The cells under pH 8 were transported more efficiently, and even more cell distribution was obtained, compared to the cells under pH 7 or pH 9 in a given time period. Up to 60% of diesel was degraded in 8 days by the *Pseudomonas* cells, which were distributed electrokinetically under the conditions of pH 8 (1,800 μS/cm, a mixture of phosphate and ammonia buffers) and 40 mA in a soil bed of 15 cm length.

Key words: Bioremediation, diesel, electrokinetic cell transport, *Pseudomonas*

There is a growing interest in the use of bioremediation to clean up the soils contaminated with organic compounds, since, in many cases of subsurface pollution, *in situ* bioremediation is the cost-effective and time-efficient treatment technology available to achieve the ultimate degradation of organic contaminants [9]. An essential factor for the successful field implementation of *in situ* bioremediation is the existence of a subsurface microbial population that can degrade the contaminants. In an environment where the indigenous microbial population is incapable of degrading the components of concern, it is desirable to introduce degradative microorganisms into the contaminated area to initiate and enhance bioremediation [24, 25]. Requiring microbial population and other key process additives such as nutrients and electron acceptors are conventionally introduced by pumping an external process fluid or by recirculating ground water through the contaminated subsurface [9]. In the absence of a favorable hydraulic permeability in soil, however, such remediation system may become biologically inactive because an effective introduction and transport of microbes or additives are often hindered by a preferential flow path, soil heterogeneity, sorption, and other complicated physicochemical interactions in the subsurface [2]. Therefore, the need for a uniform introduction of microorganisms by hydraulically driven transport processes has been a principal bottleneck in the success of *in situ* bioremediation.

One promising way to achieve a uniform and directional supply of microorganisms across the contaminated area is to utilize electrokinetics. The electrokinetic remediation process is a physicochemical remediation technique that uses direct-current electric potential differences applied across a contaminated soil mass by electrodes which are placed in the ground [1, 17, 23]. In the presence of an electric field, the transport of contaminants takes place by the mechanisms of electroosmosis, electromigration, or electrophoresis.

Earlier works in this technology have demonstrated that the electrokinetic remediation process has a great potential for removing heavy metals, small organic compounds, and NAPLs (nonaqueous-phase liquids) [1, 13, 15, 20]. It has been well suited to the removal of contaminants in heterogeneous and low-permeability soils, unlike conventional

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pump-and-treat remediation processes which rely on hydraulic injection [7, 22]. A secondary contamination of clean area by pump-and-treat technologies can be avoided since the electrokinetic technique can precisely control the flow direction through the applied electric field.

Although the electrokinetic remediation technique has been studied and applied mainly for the removal or isolation of contaminants from the soil mass, electrokinetic phenomena are also possible to be utilized in bioremediation for the purpose of supplying microbes, nutrients, electron acceptors/ donors, including other process additives like chelating agents and surfactants. Electrokinetic injection of microorganisms can be made possible since the microbial cell surface contains ionizable groups that result in an amphoteric property [4, 26]. At a neutral pH, most bacteria act as negatively charged particles, which dictates their electrophoretic movement in an electric field [4]. Therefore, strains of microorganisms capable of degrading organic contaminants can be transported with a precise directionality through soils or groundwater by electrokinetics [2].

Segall and Bruell [21] studied the feasibility of electrokinetic injection of nitrate and phosphate as nutrients to enhance biodegradation, in which nitrate supply to the soil was successful but phosphate was not transported effectively due to the precipitation with some metal components. Acar *et al.* [3] supplied ammonium hydroxide and sulfuric acid to the anode and the cathode chambers, respectively, with the intention to achieve subsoil pH control and to supply nutrients and electron acceptors simultaneously. Hydroxyl ions dissociated from ammonium hydroxide could neutralize the hydrogen ions generated from the anode, and ammonium ions were transported towards the cathode to serve as a nitrogen source. Similarly, hydrogen ions dissociated from sulfuric acid neutralized the hydroxyl ions which were generated from the cathode, and sulfate ions were transported towards the anode to serve as an electron acceptor for sulfur-reducing bacteria. DeFlaun and Condee [10] studied the transport of bacteria by applying electrokinetics to degrade some chlorinated compounds in soil, in which they noted that electrophoresis was the main mechanism of the cell transport. In the electrokinetic transport of microbial cells, a lag period can sometimes exist before the initiation of cell transport takes place through the soil, for the case of larger cells like a yeast strain [18].

To use electrokinetics for the purpose of supplying active microbes into a contaminated soil and performing biodegradation successfully, studies on the proper selection of the electrolyte condition to use should precede, because the electrolyte solution can affect the soil pH, extent of the electrokinetic phenomena, cell transport, cell activity or viability, and biodegradability. In addition, the electrolyte components used can be served as nutrients or electron acceptors/donors. The objective of this study is to investigate the effects of electrolyte buffer solutions on the electrokinetic supply of a *Pseudomonas* strain which is capable of degrading NAPLs through a diesel-contaminated sandy soil bed. The influence of different pH values, buffer components, and concentrations (or conductivity levels) was discussed regarding the maintenance of soil pH, cell transport, and resulting cell distribution, and diesel biodegradation.

MATERIALS AND METHODS

Materials

The NAPL-degrading bacterial strain *Pseudomonas* sp. OSD, isolated from a local contaminated soil in Korea, was grown at 30°C in a Luria-Burtani complex broth. The cells were harvested in the exponential growth phase by centrifugation and resuspended in the fresh broth prior to use. This strain was anticipated to be already well acclimated since it was isolated from the contaminated soil with diesel. The diesel-degrading ability of this strain was confirmed through degradation tests in a liquid phase with/ without surfactants (data not shown).

A sandy loam soil was used in this study obtained from the city of Yongin, Korea. The soil was passed through a 1.19 mm sieve, sterilized by an autoclave three times, dried at 105°C, and stored in a desiccator before use. The organic content and averaged particle density were determined as 0.37% and 2.66 g/cm^3, respectively. The organic content of dried soil was estimated by the weight loss after a 30-min heating in a 550°C furnace. The averaged particle density of soil was calculated by dividing the known mass of soil by the volume change that was observed when the soil was added to a column containing a known volume of water.

A diesel fuel oil used in this study was a commercial grade fuel for automobiles that was purchased from an LG gas station in the city of Yongin, Korea. Its specific gravity was 0.83 and the major components were known to be 75–80% of saturated linear hydrocarbons ranging from C_{20} to C_{30}. The soil was contaminated with diesel to the concentration of 4,000 mg/kg by mixing homogeneously. The homogeneity of contamination was achieved by mixing the soil with the diesel oil dissolved in an organic solvent such as acetone or n-hexane and then evaporating the solvent overnight at 40–50°C. The contaminated soil was packed and consolidated in an experimental apparatus, and a week of aging period was allowed before the experiments.

Experimental Apparatus and Set-Up

The contaminated soil was packed in a rectangular top-opened container and allowed to consolidate to make the bed of packing density 1.67 g/cm^3 and of void fraction 0.38 (Fig. 1). Details of the apparatus and experimental system

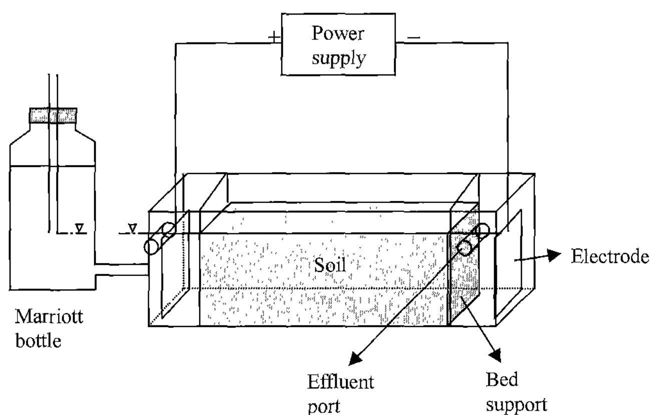

Fig. 1. Schematic experimental system of electrokinetic cell transport and bioremediation.

were described earlier [18]. The container was constructed with plexiglass (length 20 cm×depth 8 cm×width 6 cm), which is divided into three compartments: anode chamber (2.5 cm), soil chamber (15 cm), and cathode chamber (2.5 cm). To prevent the collapse of soil mass into the electrode chambers and to allow the free permeation of fluid and ionic species, an inert porous polypropylene sintered sheet with 30 μm nominal pore size was positioned between the soil bed and each electrode chamber. A graphite plate (5 cm×5 cm) of 2 mm thickness was placed in each electrode chamber as an electrode. The ends of the electrode were connected to the poles of a DC power supply (E-C Apparatus Corp., Model EC570) to create an electric field by applying a constant electric current.

Before carrying out the electrokinetic experiments, 80 ml of sterilized buffer solution of a specific pH and conductivity was filled in both electrode chambers and a Mariotte bottle containing the same buffer solution that was connected to the anode chamber. Then, a bed saturation with buffer solution was allowed for at least one day. The use of a Mariotte bottle was primarily to maintain a constant moisture level in the entire bed and to replenish the pore fluid, which might be lost due to the electroosmotic flow. The meniscus in the Mariotte bottle was adjusted, so that no flow was caused by hydraulic gradient in the soil bed. Therefore, fluid flow in the soil bed was generated only by electroosmosis and the solution level in electrode chambers was instantly recovered from the Mariotte bottle. An overflow from the cathode chamber was collected through ports in the electrode chambers. Different buffer solutions were used depending on the pH of interest: phosphate buffer for pH 7, carbonate or ammonium buffer for pH 9, and a mixture of phosphate and ammonium buffers for pH 8. A typical composition for the phosphate buffer of pH 7 was Na_2HPO_4:NaH_2PO_4= 6:4. For the carbonate buffer of pH 9, Na_2CO_3:$NaHCO_3$= 1:9, and for the ammonia buffer of pH 9, NH_4OH:NH_4Cl= 2:8. To make the pH 8 buffer, the above prepared phosphate buffer and ammonia buffer were mixed with the ratio of 3.5:6.5. For the experiments to compare the effects of buffer pH on cell transport and diesel degradation, all buffer solutions were prepared to have the same conductivity.

For undergoing the electrokinetic experiments, a constant current of mA level was applied and all operations were conducted in a horizontal configuration. The electric current of 40 mA corresponds to the current density of 0.83 mA/cm^2. When pH control in the soil bed was required during the electrokinetic experiments, the solution in each electrode chamber was changed continuously to fresh ones by using peristaltic pumps and additional buffer reservoirs.

Electrokinetic Cell Transport and Analyses

For carrying out the electrokinetic transport of bacterial cells through the soil bed (length 15 cm), *Pseudomonas* sp. OSD cells were inoculated mid-deep into the bed at a spot 4.5 cm away from the cathode chamber by injecting the suspension containing concentrated cells with a sterilized syringe. Then, a constant electric current was applied. To examine the distribution of the cell population across the bed after a specific time of electrokinetics, pore fluids in the bed were sampled at five spots with distances of 1.5, 4.5, 7.5, 10.5, and 13.5 cm from the cathode chamber (0.1, 0.3, 0.5, 0.7, along with 0.9 as a normalized distance from the cathode). In addition, CFU (colony forming unit) was counted by plating on the LB-agar plate and incubating at 30°C for three days. In a given set of experimental conditions, a percent of cell population at a specific spot was determined by dividing the CFU value at the spot by the total of CFU values which were obtained from all the five spots.

After a cell distribution was achieved across the diesel-contaminated soil by applying electrokinetics, the electric current was switched off, and then the microbial degradation of diesel was allowed by incubating the bed at an ambient condition for several days. Control experiments without the bacterial cells were also carried out to examine the possible loss of diesel from the soil bed by natural evaporation. Five grams of soil including the pore fluid were sampled at several locations in the bed and the remaining diesel oil was extracted by sonication (Ikasonic Model U50, Germany) after mixing with 15 ml of n-hexane. The diesel content in the extract was determined by a gas chromatograph (Hewlett-Packard 6890) that was equipped with an HP-5 capillary column of phenylmethysiloxane stationary phase and a flame ionization detector. Since diesel is a multicomponent mixture of hydrocarbons with various sizes, the diesel concentration was quantified by comparing the total peak area of a chromatogram with a calibration plot which was obtained from various concentrations of pure diesel oil extracted by the same procedure as indicated above.

RESULTS AND DISCUSSION

Stabilization of Soil pH

Most bacteria act as negatively charged particles at neutral or alkaline pH due to the amphoteric character of the cell surface, therefore, they can acquire electrophoretic mobility towards the anode in the electric field [4, 14]. Also, it is well known that a pH gradient is formed in the soil when electric current is applied for a long duration, since electrolysis products penetrate through the soil bed [1, 13, 15, 20], and this pH change can actually influence the microbial viability as well as the microbial transport.

As a preliminary test to study the pH change in the soil bed under an electric field and its effect on the cell transport, we inoculated the *Pseudomonas* sp. OSD cells in the soil bed that was presaturated with pH 7 phosphate buffer with 1,800 μS/cm conductivity. This strain most likely had an isoelectric point lower than pH 7 and would be negatively charged at the neutral pH, since most of the OSD cells moved towards the anode in the neutral pH region throughout all experiments. After the inoculation, the constant current of 40 mA (0.83 mA/cm^2 as current density) was applied for 24 h without any removal or addition of the electrolyte solutions in electrode chambers.

During the current application, an electroosmotic flow of 23 ml/day was observed towards the cathode and the electric potential gradient varied from 1.30 to 1.05 volt/cm. Figure 2 shows the resulting cell distribution across the bed, where the abscissa represents a normalized distance from the cathode and the ordinate for the percent of cell population. The percent of cell population was determined by dividing the CFU value at a spot in the soil bed by the total number of CFU values which were obtained at 5 spots of the distances of 1.5, 4.5, 7.5, 10.5, and 13.5 cm from the cathode (0.1, 0.3, 0.5, 0.7, and 0.9 as normalized distances from the cathode). All 100% of the cells, corresponding to 2–3×10^7 cells/ml, existed at the loading spot (normalized distance 0.3) just after inoculation. In the beginning, fractions of cells moved to the anodic direction as electric field was applied, and thus approximately 30% of the cells were found at the normalized distance of 0.5 after 9 h. However, cell transport to the anodic direction ceased thereafter, and the overall cell population in soil decreased to 10^1–10^2 cells/ml within 12 h. Most of the remaining cells were detected in the loading spot at 12 h (Fig. 2). Negligible viable CFU was found in the entire bed after 18 h. No movement of cells towards the cathode was observed during the experiments, which implied that OSD cells were negatively charged under the experimental condition used, and transported to the anodic direction by applying electrophoresis.

The major cause of cell extinction as shown in Fig. 2 would be the severe pH change in the soil bed that occurred during electrokinetics. Hydrogen and hydroxyl ions generated from the anode and cathode, respectively, transported through the soil, and thus a pH gradient was established across the soil bed, although buffered electrolyte solutions were used in both electrode chambers (no circulation data in Fig. 3). The pH around the cathode went up higher than 12 and was lowered to below 2 near the anode chamber. The whole bed would eventually be acidified if the application of electric current continued because the mobility of hydrogen ion is faster than that of hydroxyl ion in the electric field [1, 20]. Microbial viability would seriously be decreased in those extreme pH values near the electrodes and in the acidified environment.

To prevent pH change in the soil bed during electrokinetics, the electrolyte solution in each chamber was recirculated continuously with a fresh one at the rate of 80 ml/h, which corresponds to the change of one working volume of electrode chamber every hour. Figure 3

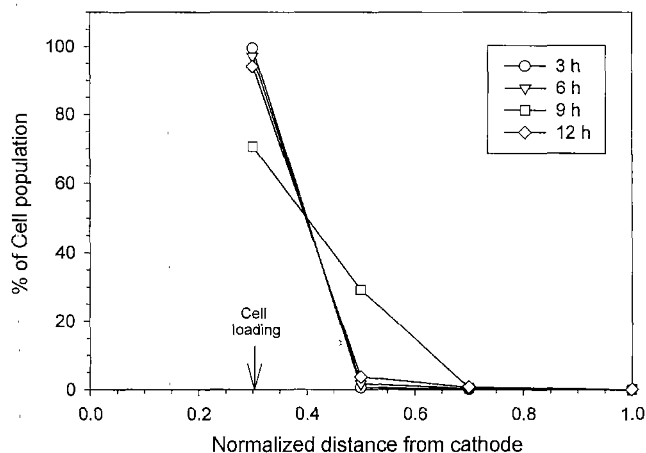

Fig. 2. Electrokinetic cell transport under pH 7 phosphate buffer and 40 mA with no amendment of the electrolyte solution.

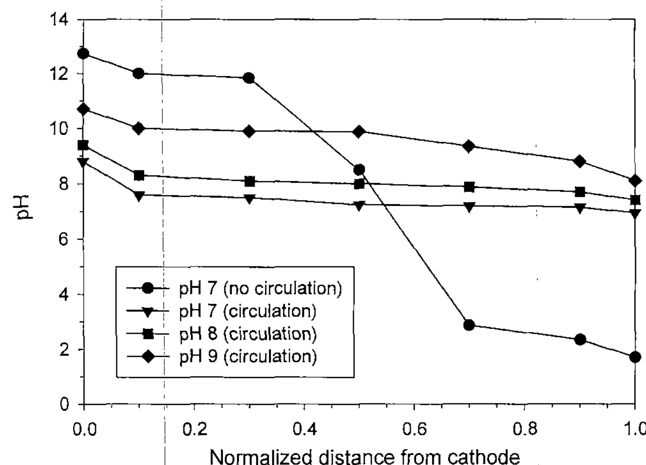

Fig. 3. Development of a pH gradient in soil bed during 24 h of 40 mA application.

shows pH profiles in the soil bed that were developed after 24 h of applying 40 mA of electric current. Different buffer solutions with the same conductivity (1,800 μS/cm) were used: phosphate buffer for pH 7, carbonate or ammonium buffer for pH 9, and a mixture of phosphate and ammonium buffers for pH 8. It can be seen that the soil pH was greatly stabilized, compared to the pH profile, without any amendment for the electrolyte solution. The most fraction of soil bed was maintained at the pH value similar to the pH of the electrolyte solution that was used in the cases of pHs 7 and 8. Therefore, soil pH was stabilized successfully by recirculating the exhausted electrolyte solution at a rate of 80 ml/h in all the later electrokinetic experiments. In the case of pH 9, two different buffers were tested and the data presented in Fig. 3 for pH 9 was obtained with carbonate buffer. Even though recirculated, ammonia buffer resulted in a severe pH gradient in soil, because the buffering strength of ammonia buffer was much lower than those of phosphate or carbonate buffers with the same conductivity: Carbonate buffer had enough buffering strength to maintain the soil pH between 9–10.

Cell Transport at Stabilized pH

The stabilization of soil pH by recirculating the electrolyte solution enabled us to transport and distribute the bacterial cells successfully across the soil bed. The bed was first saturated with pH 7 phosphate buffer of 1,800 μS/cm conductivity, and an electric current was applied for 24 h while the electrolyte buffer was recirculating at a rate of 80 ml/h. Figure 4 compares the resulting cell distributions under two different current values of 40 mA and 80 mA. Cells transported towards the anode from the loading spot and the cell population near the anode increased up to 18–20% after 24 h. Here, 100% of the cell population corresponds to about $2.2\text{–}2.5\times10^7$ cells/ml of the pore fluid. The rate of cell transport increased only slightly when a higher magnitude of electric current was applied, and the extent of increase was not proportional to the change of the electric current. This may be due to the increased extent of electroosmosis towards the cathode at a higher electric current condition. The shape of the pH profile developed in the soil bed under 80 mA was similar to that developed under 40 mA, as shown in Fig. 3.

As seen in Fig. 4b, some fraction of the cells was transported towards the cathode under 80 mA. The reason for the cell transport to the cathodic direction at a higher current was due to increased electroosmosis. The measured average electroosmotic flow rates at 40 mA and 80 mA were 20–30 ml/day and 35–54 ml/day, respectively. The directions of electroosmotic flow and electrophoretic cell movement were opposite to each other, and the cells were possibly mobilized by electroosmotic convection along with electrophoresis. Although both electrophoretic cell movement and electroosmosis were enhanced at 80 mA,

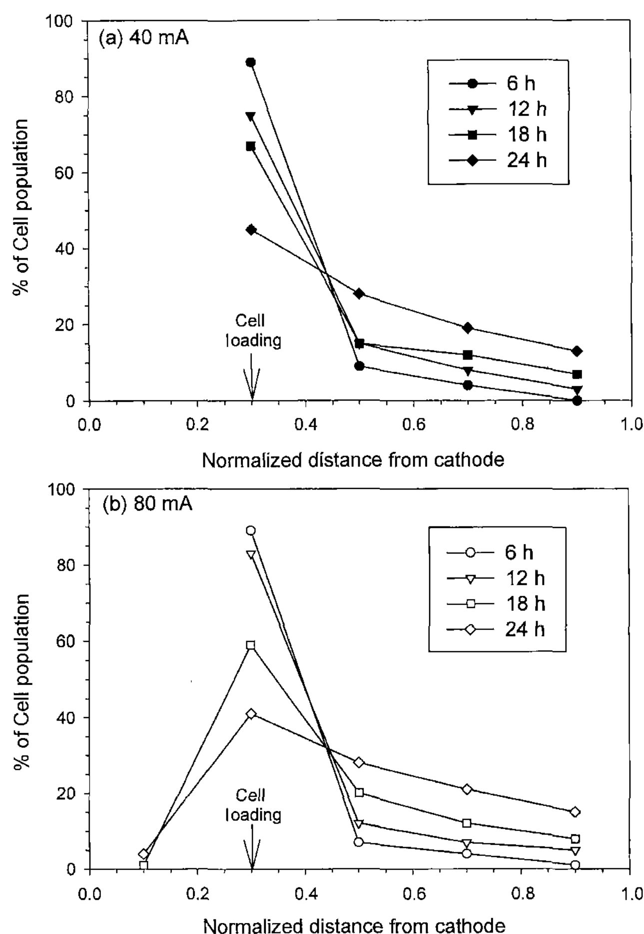

Fig. 4. Electrokinetic cell transport under two different magnitudes of applied electric current, (a) 40 mA and (b) 80 mA.
Electric current was applied for 24 h. Electrolyte solutions of pH 7 phosphate buffer with 1,800 μS/cm conductivity were recirculated at the rate of 80 ml/h.

the extent of increase of cell mobilization to the cathode by electroosmotic flow appeared to be relatively larger than the extent of increase of electrophoretic cell mobility to the anode when a high electric current was used.

Electrolyte Buffer Concentration

The effect of varying electrolyte concentrations on electrokinetic cell transport was examined. Phosphate buffer solutions of three different concentrations at pH 7 were prepared: 0.02 M (700 μS/cm), 0.05 M (1,800 μS/cm), and 0.08 M (3,000 μS/cm). Figure 5 shows the results of cell distribution by using different electrolyte concentrations after 24 h of 40 mA application. The buffer solutions in electrolyte chambers were recirculated at the rate of 80 ml/h as before. Buffer concentration of 0.05 M exhibited the best cell transport among the three concentrations and 0.02 M was found to be worse than 0.08 M. In Fig. 5, 100% of the cell population corresponds to approximately $5.2\text{–}5.7\times10^7$ cells/ml of the pore fluid.

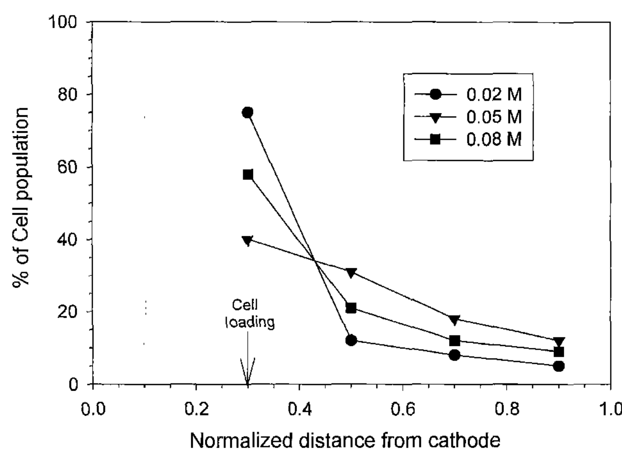

Fig. 5. Effect of the concentration of pH 7 buffered electrolyte solution on electrokinetic cell transport. Forty mA was applied for 24 h and electrolyte solutions were recirculated at a rate of 80 ml/h.

Increasing electrolyte concentration will result in two different effects in the system: an increase of electric conductivity in the pore fluid and an increase of the buffering strength. The change of conductivity may influence the electrophoretic mobility of bacterial cells, and the higher buffering strength will enhance the extent of stabilization of soil pH. A judicious selection of electrolyte concentration for an efficient electrokinetic cell transport is necessary because a higher electrolyte concentration increases the pH stability level in soil, but lowers electrophoretic cell mobility.

Effects of pH on Cell Transport and Diesel Degradation

It is known that the electroosmotic flow is independent from the electrolyte concentration in the pore water, but dependent on the surface conductivity or zeta potential of soil particle [14]. The electrophoretic mobility of microbial cell in soil can be influenced by various factors such as bulk electrolyte concentration or conductivity, charge density of cell surface, pH value relative to isoelectric point, cell hydrophobicity or the extent of cell adsorption to soil, etc. According to Smoluchowski's theory on electrokinetic phenomena and previous studies conducted in isolated environments, an absolute value of electrophoretic mobility of a colloid particle in the pH above its isoelectric point generally increases with increasing surface charge and decreases with increasing electrolyte concentration [26]. Also, electrophoretic mobility tends to increase with increasing pH, although it can decrease slightly at extremely high pH values.

An electrokinetic cell transport was carried out by using electrolyte buffers at three different pH values: pH 7 of phosphate buffer, pH 9 of carbonate buffer, and pH 8 of the mixture of phosphate and ammonium buffers. All buffer solutions were prepared to have an identical conductivity value of 1,800 μS/cm. An electric current of 40 mA was applied for 24 h after cell inoculation, and the resulting cell

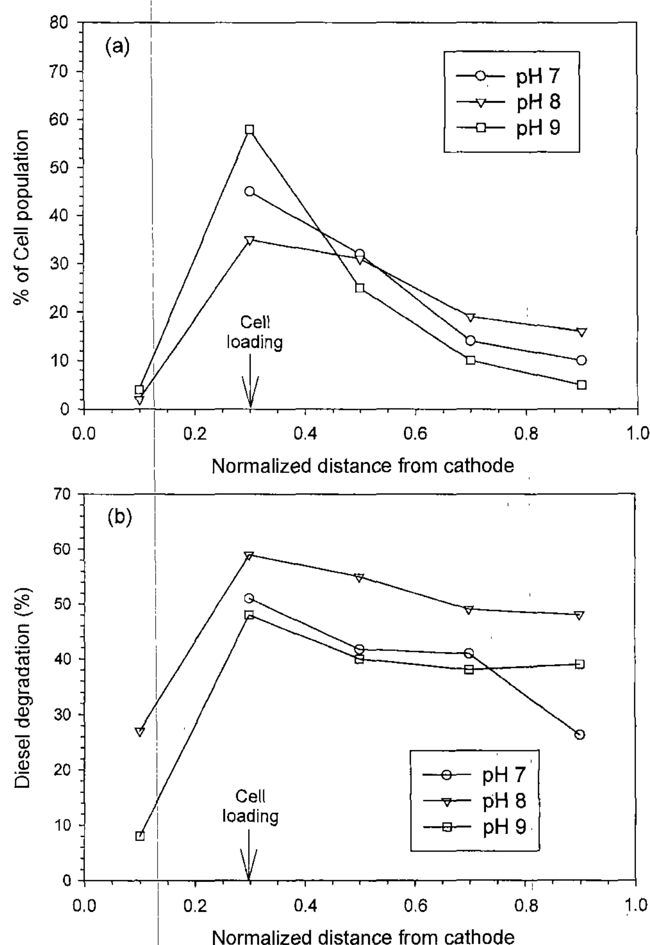

Fig. 6. Effects of electrolyte pH on the (a) electrokinetic cell distribution after 24 h of 40 mA application and (b) diesel degradation after an additional 6 days of incubation.

distribution across the soil bed was examined (Fig. 6a). Then, an additional 6 days of incubation in an ambient condition allowed the cells to degrade diesel, and the remaining diesel content in soil was determined to evaluate the extent of biodegradation (Fig. 6b). Because we sterilized the soil before the experiments, there would be no natural degradation by indigenous microorganisms. Instead, some loss of diesel by vaporization through the open surface was expected, Therefore, a control experiment without the cell inoculation was carried out. In the control experiment, about 0.5–2% loss of diesel was observed during the incubation period. The degradation data presented in Figs. 6 and 7 are the amounts of diesel disappeared minus the amount of natural loss that was obtained from the control experiments. One hundred % of the cell population in Figs. 6 and 7 corresponds to approximately $4–6\times10^5$ cells/ml of the pore fluid.

The results in Fig. 6a show that the *Pseudomonas* sp. OSD cells under pH 8 were transported more efficiently and a broader cell distribution was obtained during a given

period than the cells under pH 7. This was quite expected, since the negatively charged fraction of cells at pH 8 was greater than that at pH 7. Therefore, the electrophoretic mobility of cells increased with increasing pH, as mentioned earlier. However, pH 9 showed a less efficient cell transport than pH 8 and even less than pH 7. The reason for a poor cell transport at pH 9 carbonate buffer is not clear, but two factors can be speculated. First, pH 9 itself could be too high to maintain normal cell viability and transport. Second, some precipitation was observed during electrokinetics and this could hinder normal cell transport and diesel biodegradation when the pore space in the bed was blocked. In this study, different buffer solutions with various amounts of N and P were used, therefore, the cell distribution would have been affected by both the pH itself and the nutrient concentration supplied from the buffer components.

Figure 6b shows the results of diesel degradation for 6 days by the electrokinetically transported OSD cells. The cells transported under pH 8 condition recorded about 50–60% degradation of diesel across the soil bed, from the loading spot to the anode. The efficiencies of biodegradation by the cells transported under pH 7 and pH 9 were in the range of 30-50%. A minor extent of electroosmotic cell transport towards the cathode and a little fraction of diesel degradation near the cathode were also observed in the cases of pHs 8 and 9. The more efficient cell transport in a given period of time at pH 8 (Fig. 6a) was considered as a major contribution to the better biodegradation at pH 8. Also, the electrolyte components of pH 8 would have played a positive role in enhancing the cell growth and metabolic activity, since pH 8 electrolyte was composed of both phosphate and ammonia.

Figure 7 represents the progressive change of diesel degradation with respect to remediation time by the cells which were transported for 24 h at pH 8 and 40 mA.

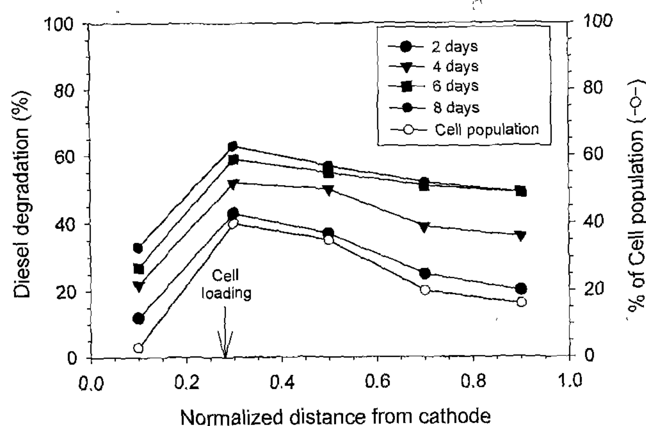

Fig. 7. Diesel degradation by the bacterial cells which were transported for 24 h under 40 mA and pH 8.

Diesel degradation was gradually increased up to 60% for the first 6 days, but there was little improvement in the degradation thereafter. The reasons for no further increase of diesel biodegradation could be explained by the following assumptions. First, nutrients became scarce after a long period of incubation, although pH 8 electrolyte buffer contained both phosphate and nitrogen. Therefore, intermittent supply of nutrients and trace elements, hopefully by electrokinetics, would be required [6, 11]. The second possibility is the limitation of bioavailability of strongly adsorbed diesel components onto the soil surface. If the cell hydrophobicity or emulsifying activity of the bacterial strain was not sufficiently high, solubilized diesel became insufficient and the available carbon sources would be subsequently limited [16, 27, 29]. One option to resolve the bioavailability problem is the use of biocompatible surfactants. Bioremediation enhanced by surfactants has been studied extensively [8, 12], and a few applications of the electrokinetic supply of surfactants for bioremediation have been reported [5, 15]. Third, available dissolved molecular oxygen would be limited to aerobic microorganisms as cell population and metabolic activity increased. The use of ORC (oxygen releasing compounds) such as peroxides is under consideration to stimulate the growth of aerobic microorganisms and to maintain biodegradation [19, 28].

Acknowledgments

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