Mobilization of *Pseudomonas putida* in Kaolin Soil by Electrokinetic Bioremediation Technique

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Abstract. Contaminated soils are due to several factors which are caused by the removal of toxins, waste from industrial activities, heavy metals from landfill and organic and inorganic waste from fertilizer used by farmers. This study aims to measure the mobilization of *Pseudomonas putida* cells through kaolin clay soil using elctrokinetic bioremediation technique. The electrokinetic is used to generate currents into the soil, thus migrating ions to opposite charge of electrodes. The *Pseudomonas putida* and distilled water was supplied into the anode and cathode reservoirs, respectively. The electrokinetic bioremediation testing was conducted for 5 days duration using 50 V of electric current. The bacterial counts of *Pseudomonas* sp. are enumerated high at $1.3x10^7$ cfu/gww (near the anode), $5.0x10^6$ cfu/gww (in the middle) and $8.0x10^6$ cfu/gww (near the cathode). These results showed high survivability of *Pseudomonas* sp. until Day 5. It was demonstrated that the electrokinetic bioremediation technique can be used for *Pseudomonas putida* transportation in kaolin clay soil throughout the soil specimen and moving in same direction with the electroosmotic flow in electrokinetic bioremediation system.

Introduction

Bioremediation harnesses the action of natural organisms in degrading unwanted chemicals. Almost all chemical contaminants, natural and man-made, are susceptible to degradation by bacteria or fungi, or even higher organisms such as plants (although often this apparent degradation will be due to microbial communities associated with the plant), given the correct conditions. The bio-availability chemical in soil describes how accessible it is to degrade organism and many factors play in role in determining this [1]. Currently, electrokinetic bioremediation technique is being employed in the contaminated land industry in some developed countries, but it is not yet implemented in Malaysia. Electrokinetic bioremediation has the advantage of being applicable in situ, meaning that excavation is not necessarily required. While for bioremediation technique, it may be difficult to achieve a satisfactory result especially when dealing with high permeability of soil. In addition, it is time-consuming, and can take months or even years to complete [2].

However, together, as electrokenetic bioremediation, it will be an extremely manageable technique providing efficient ability to treat difficult to treat areas. Moreover, it has been proposed that it may even be used to clean up soils underneath existing buildings without disturbing the foundations, due to its directional nature [3]. In fact, Li et al. [4] suggested using electrokinetic bioremediation technique can be used to treat petroleum pollutant in soil. They explained that electrokinetic accelerated the biodegradation in soil's stronger field intensity near the electrodes and the field intensity stimulated the bacterial activities, and accelerated the petroleum removal efficiency.

Even though natural soil bacteria such as *Pseudomonas* can be present in dormant or slow growing state, but when stimulated by optimum environmental conditions, they multiply rapidly and then adapt to a new environment [5,6].The microbial growth is one of the requirements of the

biodegradability of soil pollutants [7]. Therefore, this study aims to evaluate the mobility of *Pseudomonas putida* through kaolin clay soil using electokinetic bioremediation technique.

Materials and Methods

Development of Experimental Apparatus. Schematic diagram of physical modeling is presented in Fig. 1 where two small electrolyte compartments were designed to be separated from main compartment. To perform the test, kaolin soil was placed on a solid base plate in the main compartment then the electrolyte with culture broth of bacteria *Pseudomonas putida* was introduced into the anolyte compartment. Both facing of internal wall between the small electrolyte compartment and main compartment are perforated with small holes (approximately 4 mm diameter) and covered by a perforated electrode plates made of aluminum to ensure the electrodesmade good contact with the soil. The hole in both walls allowed the electrolyte fluid to flow into the soil specimen (main compartment), and also enabling bacteria *Pseudomonas putida* to flow through the system. Filter papers were attached between the perforated perspex walls and electrode plates which is sufficient to permit the passage of culture broth under working hydraulic gradient(i.e. when the electrolyte compartment was filled with bacteria *Pseudomonas putida* solution) and also allowed culture broth to flow due to external forces (under electrical gradient).

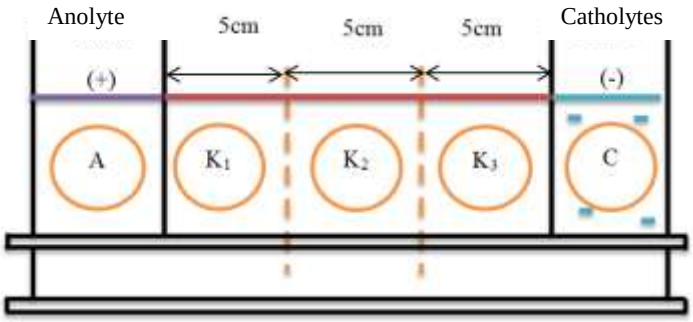

Fig. 1: Cross-section of the physical modeling of the electrokinetic cell

Table 1: Description of electrokinetic bioremediation with coding

Section (code)	Description
A	Anolyte compartment
K_1	Kaolin soil close to theanode
K_2	Kaolin soil in the middle of the specimen
K_3	Kaolin soil close to thecathode
C	Catholyte compartment

Soil Specimen Preparation. A consistent method of soil specimen preparation was employed throughout the work. It was decided from the outset to prepare soil specimens prior to contamination, in order to ensure that a realistic soil structure was presented before zinc contaminant was added. The soil sample was dried for at least three days and was ground, and passed through a 2 mm of aperture sieve size. The dry soil was mixed thoroughly with deionized water to achieve moisture content of 52 % similar to liquid limit value. The mixture was then allowed to homogenize overnight.

***Pseudomonas putida* Broth Culture**. Pure *Pseudomonas putida* ATCC 49128 samples were purchased from United States of America (USA) in dry culti-loop form. They were stored in chiller at temperature of 4 ºC prior to cultivation procedure. Approximately 16g of nutrient broth was transferred into 2500 mL Erlenmeyer flask containing 2000 mL of distilled water. The mixtures were stirred gently and then dissolved by heating on hot plate at temperature of 100 ºC for about 2

hours until complete dissolution. After that, the medium was sterilized in autoclave at temperature of 121 ºC for about 15 minutes. It was then allowed to cool down for a few minutes until its temperature drop down to around 37 ºC and 35 ºC. One loop shaft was removed from the handle straight into the medium following the manufacturer's instruction. The mixtures were then stirred until homogeneous and kept in the chiller at 4 ºC for 48 hours prior to further inoculation procedures.

***Pseudomonas putida* Cell Mobilization by Electrokinetic.** The inert aluminum electrodes for anode and cathode were prepared by the exact dimension withholes at the grid section of the plate. The size of the electrode is 19.5 x 20.0 cm each. Filter paper (47 micrometer of pore size) was used and pasted it at the wall by using distilled water before the electrode plates are slot in. The contaminated soil sample was placed into the main compartment. Nutrient broth with bacteria was poured into anolyte compartment and distilled water was poured into the catholyte compartment.

Preliminary Testing of Untreated Soil. Several tests were conducted on the kaolin clay before running the electrokinetic bioremediation test such as specific gravity, Atterberg limits and soil pH. These tests were conducted specifically to determine the characteristics of kaolin clay.

Main Testing of Treated Soil. This experiment was conducted with two different methods which is suitable for sample with bacteria (electrokinetic bioremediation) and without bacteria (electrokinetic remediation). Electrokinetic bioremediation (main testing) was conducted using *Pseudomonas putida* at the anolyte compartment and distilled water at the catholyte compartment while the electrokinetic remediation (control test) was conducted using distilled water only for both anolyte and catholyte compartments. Both methods were conducted to identify the effectiveness and the differences between electrokinetic bioremediation and electrokinetic remediation methods. However, only the results of electrokinetic bioremediation method are presented in the paper to know the mobility of *Pseudomonas putida* through kaolin clay soil by using this technique except for preliminary testing. After the electrokinetic bioremediation test was conducted, bacterial analysis was conducted for *Pseudomonas putida* – DW test using plate counts and soil microbial respiratory methods. Fig. 2 shows the electrokinetic bioremediation testing using *Pseudomonas putida* and distilled water at the anolyte and catholyte compartments, respectively.

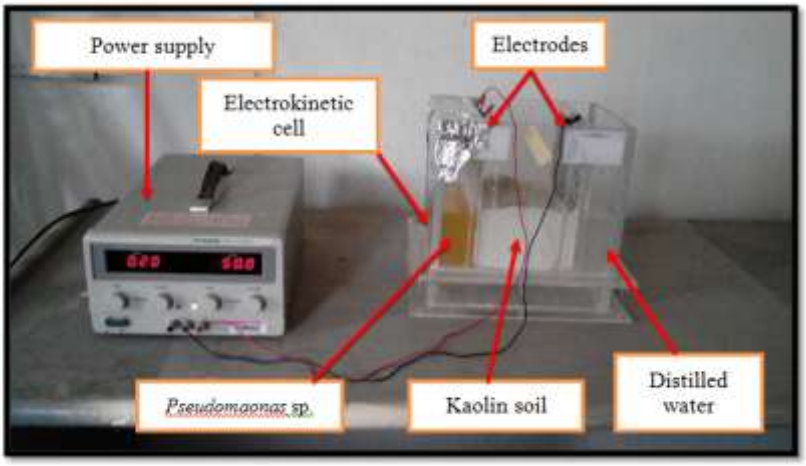

Fig. 2: Electrokinetic bioremediation testing

Results and Discussions.

The initial characteristics of kaolin soil were taken before the electrokinetic bioremediation and electrokinetic remediation technique were conducted. The plastic and liquid limit recorded percentages of 36.25 % and 52 %, while the specific gravity value is 2.58. The pH values for both experiments using bacteria and without bacteria shows lightly similar results as shown in Table 2. The pH at the initial testing for sample treatment is 4.6 while the pH at the control testing is 4.5. Both the initial and control pH of the soil are acidic. After the 5 days treatment, the results for both samples increase to pH 5.4 for electrokinetic bioremediation and pH 5.8 for the electrokinetic remediation.

Table 2: pH values of the kaolin soil before and after treatment.

pH Value	Electrokinetic bioremediation (bacteria and distilled water)	Electrokinetic remediation (both using distilled water)
Before experiment	4.6	4.5
After experiment	5.4	5.8

Bacterial count method was conducted on the kaolin clay across the soil sample and nutrient broth after the experiment was completed to ensure that the bacteria in good condition. Results shown in Fig. 3 represent the mobilization and bacteria count analysis. At the anolyte compartment (A) recorded a value of $1.0x10^8$ cfu/gww, whereas K_1, K_2, and K_3 recorded bacterial counts of $1.3x10^7$ cfu/gww, $5.0x10^6$ cfu/gww and $8.0x10^6$ cfu/gww, respectively. According to Gill et al.[8], electrokinetic enhance bioremediation by making bioaccessible contaminants, nutrients, electron acceptors and electron donors more bioavailable to catabolically active microorganisms. Microbe distribution is influenced by cell surface charge and tendency for attachment to sediments, where increase contaminant transport relates to the water partition coefficient of the specific compound; more soluble compound have greater mobility under an electric field. When an electric field is applied, the *Pseudomonas putida* migrates from the anode to the cathode electrode. The bacteria count showed that before the electrokinetic bioremediation process was conducted, no *Pseudomonas* sp. was found in the kaolin soil sample. Microbial transport research has been carried on primarily in saturated soil system, simulating either horizontal movement through an aquifer or vertical water movement [9]. Previous studies indicated that water flow is the main contributor to bacteria movement in soil and is prevented by the size of the soil pores. If the soil pores is smaller than the cell, passive diffusion of the microorganism is not possible [10].

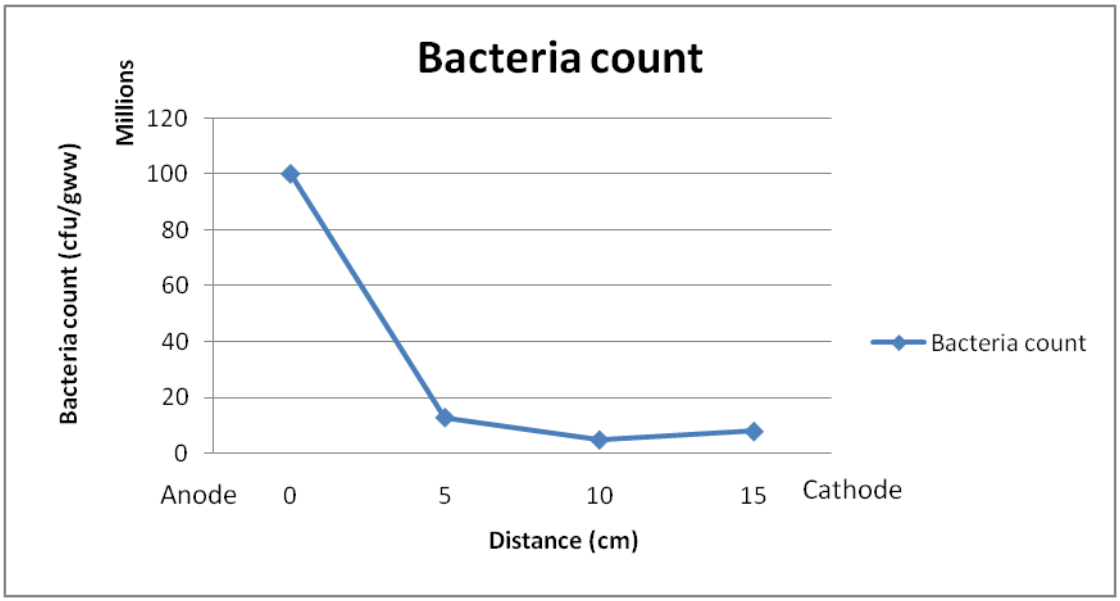

Fig. 3: The bacterial counts of *Pseudomonas putida* mobilization through the electrokinetic bioremediation technique.

Conclusion

Electrokinetic bioremediation is an effective coupled technology for contaminants removal, working through the combined effects of bioremediation and electrokinetic. Undera direct current electric field, *Pseudomonas putida* are greater near to the anode. This is mainly caused by changes in pH and a series of electric field effects. Furthermore, this technology can lengthen the growth time of the *Pseudomonas putida* and at the same time supportthe amount of bacteria greatly. As a conclusion, the study shows promising results and good indication of effect through green remediation technology by electrokinetic bioremediation technique.

Acknowledgements

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