

Electromigration of Contaminated Soil by Electro-Bioremediation Technique

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Abstract. Soil contamination with heavy metals poses major environmental and human health problems. This problem needs an efficient method and affordable technological solution such as electro-bioremediation technique. The electro-bioremediation technique used in this study is the combination of bacteria and electrokinetic process. The aim of this study is to investigate the effectiveness of *Pseudomonas putida* bacteria as a biodegradation agent to remediate contaminated soil. 5 kg of kaolin soil was spiked with 5 g of zinc oxide. During this process, the anode reservoir was filled with *Pseudomonas putida* while the cathode was filled with distilled water for 5 days at 50 V of electrical gradient. The X-Ray Fluorescent (XRF) test indicated that there was a significant reduction of zinc concentration for the soil near the anode with 89 % percentage removal. The bacteria count is high near the anode which is 1.3×10^7 cfu/gww whereas the bacteria count at the middle and near the cathode was 5.0×10^6 cfu/gww and 8.0×10^6 cfu/gww respectively. The migration of ions to the opposite charge of electrodes during the electrokinetic process resulted from the reduction of zinc. The results obtained proved that the electro-bioremediation reduced the level of contaminants in the soil sample. Thus, the electro-bioremediation technique has the potential to be used in the treatment of contaminated soil.

Keywords: Soil contamination, electro-bioremediation, biodegradation.

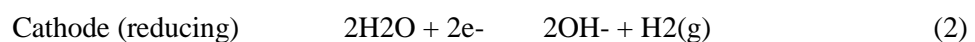
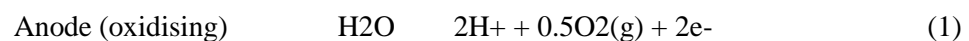
1. Introduction

Environmental contamination caused by human activities has become a serious issue worldwide. It involves contamination in various mediums such as soil, water and air. The contamination is mainly due to the large number of industrial activities, disposal of municipal solid wastes, urbanization activities and agricultural waste [1]. Currently, governments are trying to minimize the adverse impact of contamination which generally affects physical and mental development in humans, even at the lowest level of exposure [2]. Nowadays, over 80% of hazardous wastes come from industrial activities [3]. Sludge, heavy metal oil and other hazardous wastes are found in abundance. Among these hazardous wastes, heavy metal (e.g. copper, zinc, cadmium and lead) contamination is considered to



be the worst due to their harmful effects and long-term persistence in the environment [4]. Therefore, remediation of these contaminated soils becomes a great concern for both engineers and researchers.

Currently, there are several remediation methods that have been implemented. One of the promising methods is electro-bioremediation technique using microorganisms (e.g. yeast, fungi or bacteria) to degrade or remove the contaminants [5]. Electro-bioremediation is the combination of two technologies which are electrokinetic remediation technique (EK) and biological remediation. Generally, EK is a new remediation technique which mainly applies voltage at two sides of the soil and then forming electric field gradient. When electrolysis occurs in the sample, the oxidation and reduction process take place simultaneously and produce hydrogen (H⁺) and hydroxyl (OH⁻) ions. The equations representing the oxidation and reduction process are shown below [6]:



According to Syakeera et al. [7], this technique involves applying an electric current across the soil mass to boost the chemical migration from the injection point in order for it to react beneficially with the soil to bring improve its properties. On the other hand, biological remediation is a process of changing the physical and chemical characteristics through migration and the transformation process of contaminants by microorganisms. The selection of bacteria is due to the efficiency of bacteria in removing contamination found in soil [5]. *Pseudomonas putida* bacteria is naturally found in soil and can be present in dormant or slow-growing state [8]–[14].

Therefore, this study was conducted to investigate the ability of bacteria to remove heavy metals particularly zinc through the electrokinetic process. The aim of this study is to evaluate the effectiveness of bacteria as a medium to degrade and remove zinc in contaminated soil. minimize the time consuming, large data coverage and minimal ground destruction need to be discovered.

2. Materials and Methods

2.1 Soil sample preparation

Kaolin (Malaysia) Sdn. Bhd. supplied synthetic soil as a sample. To make synthetic soil, approximately 5kg of kaolin soil was mixed with 5g of zinc for 5 days. The soil sample took three days to dry. Then, the soil was grinded and sieved so that only soil particles measuring less than 2mm pass through. After that, to achieve 52% of moisture content similar to liquid limit, deionized water and soil were mixed. Lastly, the mixture was kept overnight.

2.2 Broth culture for *Pseudomonas putida* preparation

Pure *Pseudomonas putida* ATCC 49128 in dry culti-loop form was sourced from the United States of America (USA). Before the cultivation process, the *Pseudomonas putida* was stored in the chiller at 4°C. Approximately 16g of medium (nutrient broth) was filled into an Erlenmeyer flask containing distilled water and stirred. Then, the medium was heated at 100°C on a hot plate to dissolved it until complete dissolution for around 2 hours. Next, the solution was sterilized for around 15 minutes at 121°C in an autoclave machine. The solution was cooled for a few minutes around 35°C to 37°C. According to manufacturer instruction, one loop shaft was removed from the holder and put directly on the medium. Lastly, before the inoculation process, the mixture was stirred well and kept inside the chiller at 4°C for 2 days.

2.3 Bacteria counting (Spread plate method)

Firstly, nutrient media was prepared using *Pseudomonas* agar. The *Pseudomonas* agar was mixed with distilled water in the beaker and boiled. The agar was cooled up to 45 to 50°C and poured into half of the six petri plates. Then, bacteria dilution was prepared. 1mL from the sample was removed and blew

into 9mL dilution in tube 1. Then, 1mL from tube 1 was placed into the 9mL dilution in tube 2 and the procedure is repeated until tube 6. After that, the solid medium in the plate was inoculated and spread over the surface evenly. All the petri plates were placed inside the incubator for 24 hours with a temperature of 37°C. The bacteria colonies grew on the surface of the medium. The petri plate was placed on the counting chamber to keep track of the bacteria count.

2.4 Electrokinetic bioremediation testing

Figure 1 and figure 2 shows the real model and cross section of electrokinetic bioremediation testing. At the anode, the reservoir was filled with *Pseudomonas putida*. The cathode of the reservoir was filled with distilled water. Direct electric current 50V was applied at the anode and cathode for 5 days. At the end of the duration, the samples of soil were taken from K1, K2 and K3 of the anode for XRF test and bacteria count test.

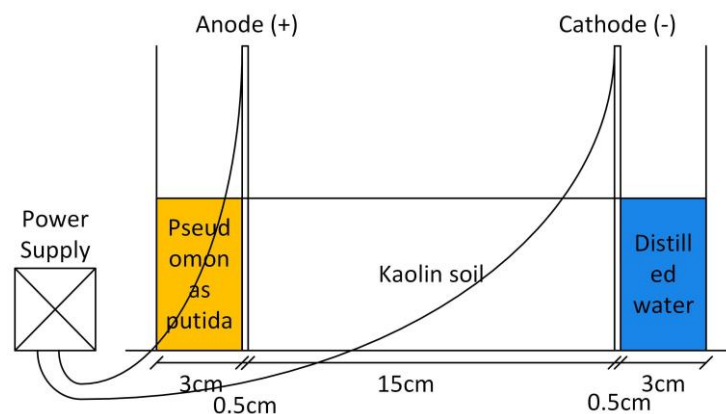


Figure 1. Electrokinetic bioremediation test.

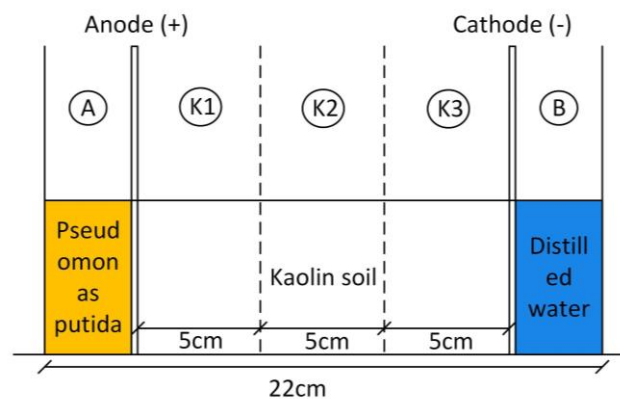


Figure 2. Cross-section of the physical modeling of the electrokinetic bioremediation tank.

Table 1. Description of electrokinetic bioremediation (code).

Section (code)	Description
A	Anode reservoir
K ₁	5cm from anode reservoir
K ₂	10cm from anode reservoir
K ₃	15cm from anode reservoir
B	Cathode reservoir

3. Results and Discussions

3.1 pH development

Due to pH sensitivity during chemical interactions, it was necessary to evaluate the pH of soil before and after treatment. It was observed that the pH values are slightly similar as shown in Table 2. From the table, it was observed that soil pH before and after treatment using bacteria and distilled water was acidic which is 4.6 and 5.4 respectively. Meanwhile, pH of soil before and after treatment using distilled water stand-alone was also acidic at 4.6 and 5.8 respectively. This significant result indicates that the presence of bacteria does not affect the pH development in soil. This statement is supported by a research conducted by Zeng et. al [15] which stated that the mobility of heavy metals increase with the decrease in soil pH.

Table 2. pH values of kaolin soil before and after treatment using and without using bacteria.

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

3.2 Concentration and bacteria

Table 3 shows the amount of concentration before and after treatment. The results indicated the different values of concentration from K1 to K3. The initial concentration of heavy metals before the treatment is different. The results indicated that there was a significant reduction of zinc concentration at K1 and K2, which was 80mg/L (89%) and 284mg/L (60%) respectively. However, the concentration of zinc at K3 increased to 1674mg/L (137%). The concentration increased to 1674mg/L because the zinc ions in the soil migrated and accumulated in this section.

The initial concentration for Pb, Cr and Cu was 255mg/L, 36mg/L and 7mg/L respectively. From the results, the heavy metals show a slight decrease at K1, which are 227mg/L (11%) for Pb, 28mg/L (22%) for Cr and 4mg/L (43%) for Cu. However, the concentration values of Pb, Cr and Cu at K2 and K3 were lower compared to zinc concentration. For this situation, the concentration of zinc was higher than the other three heavy metals because Pb, Cr and Cu have no additional chemicals added to the soil. This is because the soil sample was added with 5g of zinc oxide.

Figure 3 shows that the migration of bacteria from anolyte to K3. At the anolyte section, the recorded value is 1.0×10^8 cfu/gww. However, K1, K2 and K3 recorded values of 1.3×10^7 cfu/gww, 5.0×10^6 cfu/gww and 8.0×10^6 cfu/gww, respectively. It shows that the bacteria was still in good condition until the treatment was completed. The reduction of zinc concentration is caused by the bacteria. Duxbury [16] stated that bacteria used heavy metal as micronutrients. Overall, it was proven that the reduction of heavy metal occurs when the presence of bacteria in this treatment and the ions migrate toward the cathode [17].

Table 3. Concentration values for initial and after treatment.

	Distance from anode (cm)	Zn (mg/L)	Pb (mg/L)	Cr (mg/L)	Cu (mg/L)
Initial Concentration Before Treatment	0	706	255	36	7
Concentration After Treatment- 5cm	5cm	80	227	28	4
Concentration After Treatment – 10cm	10cm	284	233	27	1
Concentration After Treatment – 15cm	15cm	1674	231	24	2

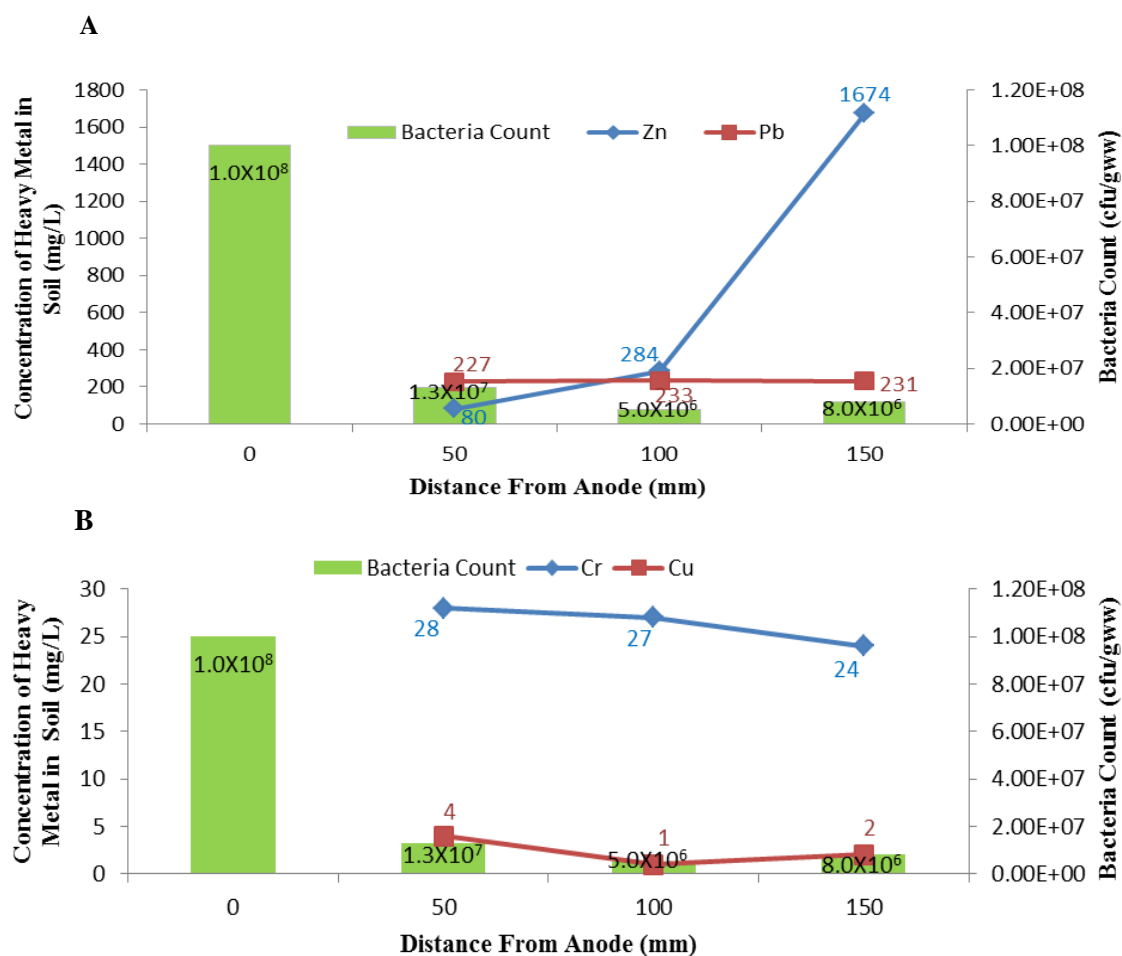


Figure 3. Bacteria migration and concentration of Zn and Pb (A) and concentration of Cr and Cu (B) after EK remediation.

4. Conclusion

Electrokinetic bioremediation is an alternative technology to remove contaminants in soil. This technology used a combination of bioremediation and electrokinetics. From this treatment and the results of zinc and bacteria count, it is proven that electro-bioremediation can remediate the contaminated soil up to 89%. This treatment only focuses on *pseudomonas putida* as an electro-bioremediation agent. Nevertheless, using various types of bacterial consortium is recommended for this treatment. To conclude, this technology is useful especially for industries to treat contaminated soil through green remediation technology.

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