Novel Technique to improve the pH of Acidic Barren Soil using Electrokinetic-bioremediation with the application of Vetiver Grass

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Abstract. Residual acidic slopes which are not covered by vegetation greatly increases the risk of soil erosion. In addition, low soil pH can bring numerous problems such as Al and Fe toxicity, land degradation issues and some problems related to vegetation. In this research, a series of electrokinetic bioremediation (EK-Bio) treatments using Bacillus sphaericus, Bacillus subtilis and Pseudomonas putida with a combination of Vetiver grass were performed in the laboratory. Investigations were conducted for 14 days and included the observation of changes in the soil pH and the mobilization of microorganism cells through an electrical gradient of 50 V/m under low pH. Based on the results obtained, this study has successfully proven that the pH of soil increases after going through electrokinetic bioremediation (EK-Bio). The treatment using Bacillus sphaericus increases the pH from 2.95 up to 4.80, followed by Bacillus subtilis with a value of 4.66. Based on the overall performance, Bacillus sphaericus show the highest number of bacterial cells in acidic soil with a value of 6.6 x 10² cfu/g, followed by Bacillus subtilis with a value of 5.7 x 10² cfu/g. In conclusion, Bacillus sphaericus and Bacillus subtilis show high survivability and is suitable to be used in the remediation of acidic soil.

Keywords: Barren acidic soils, Electrokinetic bioremediation

1. Introduction

Nowadays, the erosion of residual or barren slopes has become a serious problem and some areas contain soil which is highly acidic. Low soil pH brings numerous potential related problems including toxicity of soil, land degradation issues and some problems related to vegetation [1]. Furthermore, the soil acidity will directly affect plant growth. Thus, the natural rates of soil erosion are lower for soil with a good cover of vegetation compared to bare soil [2]. In fact, the residual acidic slopes which are not covered by vegetation greatly increase the risk of soil erosion [3]. Normally, slope failures or soil erosion can bring damage to human lives and cause destruction of property. This problem happens because the acidic slope
area is not able to support the development and growth of any vegetation. In pastures grown on acidic soils, the growth of plants will be adversely affected. Therefore, it is important to reduce the toxic elements in soil such as aluminium and iron because these elements are the main causes leading to failure of plant growth [15]. Without any treatment, acidic soil can cause several environmental problems.

Recently, there are many techniques that have been used by previous researchers to reduce the toxicity levels in soil [13,14]. In the last decade, the development of electrokinetics and its combination with bioremediation technologies have become a research focus [4]. The electrokinetic technology is a green remediation technology and has already been used for the treatment of soils contaminated by heavy metals [5].

The main focus in this research is to study the effectiveness of electrokinetic bioremediation technique where selected bacteria (*Bacillus sphaericus*, *Bacillus subtilis* and *Pseudomonas putida*) were used to remediate the acidic soil and to increase the pH of the residual acidic slope [1]. Instead of only using different types of microorganisms, this research also focuses on the use of EK-Bio with Vetiver grass in the treatment process to evaluate the effectiveness of remediation.

By applying this technique, there is a high potential of biodegrading and detoxifying acidic metals in soil as reported by Winarso et. al. [6], Panhwar et. al. [7] and Mohammadi [8] and other researchers in previous studies. Bioremediation is also a relatively simple technology compared to other techniques. On the other hand, EK-Bio is intended to activate dormant microbial populations using nutrients to promote growth, reproduction, and metabolism of the microorganisms capable of transforming soil polluted with toxic elements [9].

Another advantage achieved by bioremediation is that it will reduce the maintenance of residual soil and hillsides which are not covered by vegetation. Besides that, it is also a sustainable and affordable type of green technology which can be used in the remediation of acidic soil. This study can provide valuable information for geo-environmental engineers in order to remediate acidic soils in future.

2. Materials and Methods

2.1 Preparation of soil sample

The soil that was used throughout this study was obtained from Ayer Hitam, Johor where the sample was collected from a hillside residual area at Kampung Seri Desa Laut as shown in Figure 1 below. Disturbed soil samples were taken from the site. Approximately 80 kg of soil samples were collected from the surface at depths of 0 to 0.5 m. The collected soil samples were then put into clean containers. Table 1 shows the initial physical and chemical characteristics of the soil.

![Figure 1. Sampling Location](image)
Table 1. Initial physical and biological characteristics of soil

| Soil property                        | Value  |
|--------------------------------------|--------|
| Plastic limit (%)                    | 27     |
| Liquid limit (%)                     | 46     |
| Specific gravity                     | 2.70   |
| Field density (Mg/m³)                | 1.43   |
| Falling head permeability (m/s)      | 6.6 x 10⁻⁷ |

2.3 Design of Electrokinetic Treatment Tank
The electrokinetic model tank diagram was illustrated as shown in Figure 2. It was designed using Perspex glass. This system mainly consisted of an electrokinetic cell, anolyte and catholyte compartment, and power supply. The electrokinetic cell was divided into 3 sections which are the anode compartment, the cathode compartment and the main compartment which was used for treated acidic soil placement. The glass cell model tank had a dimension of 20 cm in width, 22 cm in length and 20 cm in height. Both the anolyte and catholyte compartments were separated from the main compartment and had a dimension of 3 cm in length and 20 cm in width. There were a few small holes measuring 5 mm in diameter that was drilled beforehand in both internal walls between the compartments. The small hole in both walls allowed the electrolyte fluid to flow into the samples and also enabled bacteria to flow through the system. Figure 2 shows a schematic diagram of the small holes in both walls. Filter papers measuring 47 micrometers in pore size were used and placed between the Perspex walls and electrode plates in both compartments to prevent the treated acidic soil from entering both anolyte and catholyte compartments and to stabilize the soil sample. It is important to make sure that it is sufficient to permit the passage of cultured broth containing the bacteria. In the main compartment, the wall was covered with aluminium electrode plates. This is important to ensure that the electrode makes good contact with the soil. The size of the electrode plate is 19 cm x 20 cm.

2.4 Preparation of Electrokinetic Bioremediation Test
Acidic soil samples were placed in a tray for the drying process which took 24 hours under a temperature of 105 ºC. After 1 day, the soil was grinded using a soil grinding machine before the next process. Then, the soil samples were sieved using a mechanical sieve machine. The soils that passed through the 425 μm sieve amounted to 15 kg. The soil was then placed into a clean industrial mixing machine. 6 kg of distilled water was then poured into the mixer machine and the mixer was set at a medium control speed. The slurry sample was then poured into the main compartment of the electrokinetic treatment tank. Compaction was done using a heavy load and the sample was left for one weeks before performing the
test. After the compaction process was complete, the electrokinetic bioremediation process was carried out on the sample. A constant current of 10 V was applied to the samples through a DC power supply generator that was connected to the electrodes. These experiments were conducted for 14 days. At the first stage, *Bacillus sphaericus* was used in the treatment, followed by *Bacillus subtilis* and *Pseudomonas putida*. The samples were observed and the data were recorded every 24 hours.

The anode compartment was filled with a nutrient broth containing bacteria while the cathode compartment was filled with distilled water. On both sides of the compartments, the liquid is maintained at the same height to avoid differences in hydraulic gradient. The use of distilled water and the bacteria broth was to determine the effectiveness of the treatment. Two experiments, namely electrokinetic bioremediation and electrokinetic remediation were carried out. Both electrokinetic treatments were then divided by two cases which is treatment with plants and treatment without plants as shown in Figure 3. Vetiver grass was selected to be used in the electrokinetic treatment based on its performance in phytoremediation to remove toxins as reported by previous researchers. Electrokinetic bioremediation was conducted using bacteria in the anode compartment and distilled water in the cathode compartment, while the electrokinetic remediation which is used for samples without bacteria was conducted using distilled water in both compartments. Electrokinetic remediation was used as a control test in this experiment. Cultured bacteria broth was placed in section A (anode compartment) and distilled water was placed in section C (cathode compartment). Point A at the top layer (AT), point A at the bottom layer (AB), point M at the top layer (MT), point M at the bottom layer (MB), point C at the top layer (CT) and point C at the bottom layer (CB) represent the distribution of acidic soil samples that were divided into 3 sections (anode, middle, cathode) and 2 layers per section.

![Figure 3](image)

**Figure 3.** a) EK-Bio with plants and b) EK-Bio without plants

### 2.5 Preparation of Nutrient Broth

In this experiment, Tryptic Soy Broth (TSB) powder was used as the broth media. To perform the test, approximately 30 g of TSB was mixed together with 1000 ml of pure water in a blue capped glass bottle. The broth solution was dissolved and stirred using a magnetic bar and heated using a hot plate. Then, the solvent was sterilized in an autoclave sterilizer machine for about 2 hours at a temperature of 121˚C. After that, the heated solvent was left to allow it to cool until the temperature dropped to 35 ˚C.

### 2.6 Preparation of Nutrient Agar

The preparation process of nutrient agar is quite similar to the preparation of the nutrient broth. However, a different type of media was used. Tryptic Soy Agar (TSA) was used as the nutrient media. In order to conduct this test, 40 g of TSA powder was mixed together with 1000 ml of pure water in a beaker. By referring to laboratory procedures, the solution needs to be stirred and heated using a hot plate at a
temperature of 100 °C with a speed of 200 rpm. This process has to go on until the liquid seems clear and there is no suspended solid observed. Thereafter, the solvent was placed in the autoclave machine at 15 psi, and the temperature was set at 121 °C for 2 hours. Similar to the process of making the nutrient broth, the solvent needs to be left to cool for a few minutes until the temperature reaches 48 °C before pouring the liquid into a petri dish to allow it to change to its solid form.

3. Results and Discussions

3.1 Change of pH at Electrolytes

Figure 4(a) shows the pH of the electrolyte for the control sample. The electrolyte pH ranged between 3.21 - 4.21 at the anode while the electrolyte pH for the cathode ranged between 3.52 - 3.45. The pH values for the anode electrolyte and the cathode electrolyte ranged between pH 4.16 and pH 4.21 on the first day. On the second day of the test, the pH value increased steadily until day 3. Both the electrolyte pH at the anode and the cathode rapidly decreased to approximately pH 3.21 and 3.61 on day 6. The pH slightly increased starting from day 7 until day 14 for both electrolytes. As reported by Harbottle [10], the hydrogen and hydroxyl ions would migrate into the soil through electromigration and diffusion, and later changing the pH of the soil pore fluid.

Meanwhile, Figure 4(b) shows the pH electrolyte for the treatment using Bacillus sphaericus at the anode compartment. The pH value of the anode electrolyte increased with time from approximately pH 7.23 until pH 7.80. The pH condition was considered neutral even though there seemed to be a decrease in pH on day 11. The trend of the pH value of the cathode electrolyte was similar to that of the anode electrolyte where the pH value increased from pH 4.18 on day 1 to pH 4.75. These values remained constant until the current was stopped after 14 days of treatment. The pH is an important parameter in the electrokinetic process because soil surface properties such as cation exchange capacity, adsorption capacity, sign and magnitude of zeta potential as well as speciation and dissolution of pollutants and toxicity elements are generally pH dependent.

In contrast, the pH in Figure 4(c) showed the same trend for both anode and cathode electrolytes from the beginning of the test. The anode electrolyte started with pH 5.35 on day 1 and maintained constant throughout the 14 days with a pH value of 5.35. On the other hand, the pH range of the cathode electrolyte was 3.97-4.44. In contrast with the pH values of the anode electrolyte, the pH values of the cathode electrolyte changed quite significantly due to the flow of water [11]. In order to enhance the electrokinetic remediation process, a favorable pH condition is necessary. The pH values of anode and cathode electrolyte seem to decrease on day 2 at 3.06 and 2.02 respectively, and then the pH increased until Day 4. The pH fluctuated from day 1 until day 9. After that point, the pH increased sharply to reach a maximum pH value of 4.09 for the anode electrolyte and 2.20 for the cathode electrolyte.

Figure 4(d) shows the changes in the values of pH at the anode electrolyte and the cathode electrolyte. The pH values of the anode and cathode electrolyte decreased on Day 2 with value of 3.06 and 2.02 respectively, and then the pH value increased until Day 4. The pH fluctuated from day 1 until day 9. After that point, the pH increased sharply to reach a maximum pH value of 4.09 for the anode electrolyte and 2.20 for the cathode electrolyte.

In the other hand with the pH value of the anode electrolyte, the pH values of the cathode electrolyte changed quite significantly due to the flow of water that carried some of the hydroxide ions into the catholyte compartment. Therefore, the pH of the cathode electrolyte decreased.
3.2 Changes of pH at Soil

Figure 5(a) shows the pH results for 1, 3, 7 and 14 days of control treatment. From the graph line, it was observed that the pH variation profile for all different days of treatment showed a similar pattern. The pH values of day 1 at the anode and the cathode were reduced to pH 3.38 and 3.48, respectively. Then, it increased to pH 4.19 (5 cm from anode) and 4.53 (15 cm from anode). The increasing pH values near the cathode during the 14 day treatment are due to the migration of hydrogen ions at the cathode while the decreasing pH values near the anode region are due to the migration of hydroxide ions at the cathode. In summary, based on Acar and Alshawabkeh [5], water generates H⁺ ions at the anode and OH⁻ ions at the cathode.

Results of pH distribution across the soil samples for EK-Bio treatment using *Bacillus sphaericus* showed a clear indication of electrokinetic effects on pH at both the anode and the cathode for 1, 3, 7 and 14 days of treatment as presented in Figure 5(b). The pH value for both treatments with and without Vetiver grass was significantly higher than the control value as presented in Figure 5(a). At 5 cm from the anode, the pH value started increasing from 3.72 to 3.44 on day 14 of the treatment without Vetiver grass and increased from 3.82 to 4.65 for treatment with Vetiver grass. At 15 cm from the anode, the pH values also increase from 3.84 to 4.58 (without Vetiver grass) and from 3.87 to 4.80 (with Vetiver grass). The results proved that a combination of EK-Bio treatment with Vetiver grass can increase the pH of soil.
Initially, it should be recognized that under an electric potential, the electrolysis of water occurs at the electrodes. Thus, the electrolysis reactions caused an acidic solution to be generated at the anode.

However, after a longer treatment period was applied for the tests on day 1, 3, 7 and 14, the pH of soil treated using *Bacillus subtilis* decreased from day 1 until day 3. The pH values for both treatments (without and with Vetiver grass) dropped to 3.28 and 3.12 at 5 cm from anode while the pH values decreased to 3.50 and 3.78 at 15 cm from anode. Then, the pH increased significantly from day 3 until day 14 as shown in Figure 5(c). The electrokinetic effects on pH values at the anode seemed lower than cathode. Based on Tajuddin [11], in the electrolysis process, hydrogen ions are released. It can contribute to the acidity of the environment around the anode region. This is the main reason of the reduction in pH values near the anode over 14 days. The increase in pH values at the cathode was due to the release of hydroxide ions near the cathode.

Contrary, there was a drastic pH change after 14 days of EK-Bio treatment at the anode when *Pseudomonas putida* broth and distilled water were applied at the anode and the cathode. From Figure 5(d), the pH value decreased and created a more acidic environment at the cathode. The combination of EK-Bio treatment with Vetiver grass shows better results compared to the treatment without Vetiver grass. For both treatments, the pH started to decrease on day 3 and then there was a significant increase in pH values which were 3.04 (without Vetiver grass) and 2.97 (with Vetiver grass) at the cathode on day 14 as presented in Figure 5(d).

![Figure 5](image_url)  
*Figure 5.* Changes of pH in soil with and without Vetiver a) control, b) EK-Bio using *Bacillus sphaericus*, c) EK-Bio using *Bacillus subtilis* and d) EK-Bio using *Pseudomonas putida*
Conclusion
This study has successfully proved that the pH of soil increased via electrokinetic bioremediation (EK-Bio) in combination with Vetiver grass (phytoremediation). The original pH value of acidic residual soil before treatment was pH 2.95. The treatment via EK-Bio using Bacillus sphaericus increased the pH value to 4.80, followed by Bacillus subtilis with a pH value of 4.66 after 14 days. However, the performance of Pseudomonas putida in increasing the pH of acidic soil was not very successful. The final pH was 2.98 after 14 days. In conclusion, Bacillus sphaericus and Bacillus subtilis are capable of increasing the pH of acidic soil even though the final pH was still highly acidic. Therefore, Bacillus sphaericus is suggested to be used in the electrokinetic remediation of acidic soil.

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