Assessment and Comparison of Electrokinetic and Electrokinetic-bioremediation Techniques for Mercury Contaminated Soil

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Abstract. Landfills are major sources of contamination due to the presence of harmful bacteria and heavy metals. Electrokinetic-Bioremediation (Ek-Bio) is one of the techniques that can be conducted to remediate contaminated soil. Therefore, the most prominent bacteria from landfill soil will be isolated to determine their optimal conditions for culture and growth. The degradation rate and the effectiveness of selected local bacteria were used to reduce soil contamination. Hence, this enhances microbiological activities to degrade contaminants in soil and reduce the content of heavy metals. The aim of this study is to investigate the ability of isolated bacteria (Lysinibacillus fusiformis) to remove mercury in landfill soil. 5 kg of landfill soil was mixed with deionized water to make it into slurry condition for the purpose of electrokinetic and bioremediation. This remediation technique was conducted for 7 days by using 50 V/m of electrical gradient and Lysinibacillus fusiformis bacteria was applied at the anode reservoir. The slurry landfill soil was located at the middle of the reservoir while distilled water was placed at the cathode of reservoir. After undergoing treatment for 7 days, the mercury analyzer showed that there was a significant reduction of approximately up to 78 % of mercury concentration for the landfill soil. From the results, it is proven that electrokinetic bioremediation technique is able to remove mercury within in a short period of time. Thus, a combination of Lysinibacillus fusiformis and electrokinetic technique has the potential to remove mercury from contaminated soil in Malaysia.

Keywords: Landfill soils, Electro-bioremediation, Lysinibacillus fusiformis

1. Introduction
In recent years, the disposal of solid waste at landfills has caused the degradation of land as well as the pollution of the environment. Chemicals from solid waste would be transferred to the ground and may

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remain in the soil [1]. Therefore, contaminated soil needs to be treated. According to Raghab et. al. [2] leachate treatment technologies consist of two basic steps which are biological treatment and also physical and chemical treatment. It is known that the disposal of solid waste in landfills may cause severe environmental impact if the leachate and gas emissions are not controlled. Leachate that is generated from the municipal landfill contains large amounts of organic and inorganic contaminants [19,20]. When there is a high concentration of heavy metals, it may contain some hazardous organic chemicals that are dangerous to the health environment such as mercury [3].

Mercury contamination is a very critical type of pollution that can affect humans and wildlife [4]. Normally, mercury pollution originates from anthropogenic and natural activities such as industries, mining activities and sludge dumping [5]. This type of pollution needs to be monitored to avoid it from becoming worse. Therefore, there are several techniques which are available to remediate soil contamination by mercury such as solidification/stabilization, soil washing, vitrification, phytoremediation, electrokinetic remediation and biological treatment [6,7,8,9]. From all of the techniques, this study only focuses on the combination of two technologies which are electrokinetic and biological in nature to increase the capability of these methods to remove/reduce the amount of mercury in soil.

The application of electrokinetics helps to transport the ions where the electric field is applied across the soil. Positive ions will be attracted to the cathode and negative ions will be attracted to the anode. Meanwhile, bioremediation is the process of using bacteria to lessen the toxicity levels of mercury [10]. Thus, the pollutant molecules and bacteria in the soil will be affected by the process of electrokinetic and bioremediation that had been applied [11]. Electrokinetic separation is an emerging technology that relies on the application of a low-density, direct current through the soil to separate and extract heavy metal, radionuclides and organic contaminants from unsaturated soil, sludge and sediment. This technology can be applied to contaminant concentration ranging from a small amount of ppm to concentrations greater than 10,000 ppm. However, it may not be effective for treating multiple contaminants that have significantly different concentrations.

For the most part, the application of both electrokinetics and bioremediation offers many advantages where the process of bioavailability can be solved by using electrokinetics [11]. The electric field is capable of moving the contaminant molecules by moving them out from inaccessible pores, redistributing high concentrations and also by desorbing the contaminant from the soil. In addition, the use of electrokinetics coupled with bioremediation is a procedure for remediating contaminated land which has successfully been used to stimulate the degradation of soil contaminants [12] and facilitate the removal of heavy metals [13].

2. Materials and Methods

2.1 Sampling Location and Technique

The soil samples were collected at Bukit Bakri Landfill, Muar, Johor with the coordinates 2.041317, 102.673008. In this study, the standard used was according to ASTM D5633-04 Standard Practice for Sampling with Scoop. Disturbed soil samples were collected from the site by pushing the blade of a shovel into the soil till a depth of 10-15 cm beneath the surface. A triangular wedge of soil was removed the shovel and set aside (to be replaced after sampling). After that, the blades are pushed into the soil again taking a thin (1.5 cm) slice from one side of the hole. With a knife, trim the slice to obtain a 2.5 cm strip of soil down the center of the spade-top to bottom. Take this “core” as part of the composite laboratory samples. The core samples were kept in flasks and subsequently transported to the laboratory. The soil was oven-dried at 100 °C for 24 hours, grinded using a mortar grinder and then sieved to remove plants and debris. Table 1 shows the initial physical and biological characteristics of soil.

2.2 Material and Equipment

Electrokinetic cell requires electrokinetic testing cell, stainless steel plate, power supply and wires. On the other hand, Isolated bacteria from the soil (L.fusiformis was selected based on the highest probability
available in the soil), Tryptic soy broth (Brand: Merck KGaA), Tryptic soy agar (Brand: Merck KGaA), autoclave (Model HV-85), incubator shaker, microbiological incubator, laminar flow cabinet and Mercury Analyzer (MA-3000 RD/SC).

| Soil property                  | Value  |
|-------------------------------|--------|
| Plastic limit (%)             | 32     |
| Liquid limit (%)              | 62     |
| Specific gravity              | 2.88   |
| Serpens flexibilis (%)        | 59.6   |
| Lysinibacillus fusiformis (%) | 64.6   |
| Bacillus vallismortis (%)     | 55.5   |

2.3 Electrokinetic Testing Cell

Figure 1 shows the diagram of the real setting of the test. The electrokinetic cells are made of Perspex glass tubes which is 22 cm in diameter. The untreated soil which was placed inside the electrokinetic cells was divided into 3 areas which are near anode, middle and near cathode and for every area was divided onto four sections which are 3 cm, 6 cm, 9 cm and 12 cm from the bottom of the tank as shown in Figure 2. Table 2 shows the description of electrokinetic bioremediation. Each segment/layer of soil has the same level of consolidation in order to ensure stability of flow inside the mixture of soil. 7.5 V direct electric current was supplied at the anode and cathode compartment for 7 days of treatment. The anode and cathode attract ions towards them in which the ions will accumulate/gather at the electrode itself. The anode is submerged inside the bacteria dilution in which the purpose of the bacteria itself is to remediate the untreated soil whereas the cathode is submerged inside distilled water. Both liquids were at an equal parallax height to ensure that there will be no hydraulic gradient between them.
2.4 Preparation of Nutrient Broth

Approximately 30 g of Tryptic Soy Broth was suspended in 1 L of demineralized water in Erlenmeyer flasks and the solution was stirred gently to homogenize them. The solution was then sterilized in a Hiramaya Autoclave Sterilizer for 15 minutes at 121 °C. The solution was allowed to cool down for a few minutes until its temperature dropped between 35 - 37 °C. 6 inoculated cells of bacteria _L. fusiformis_ were immediately placed into the warm medium nutrient broth. The broth was stirred for a few minutes before being stored in an incubator shaker at 35 °C for 9 days (colour changes from amber to dark amber).

2.5 Preparation of Nutrient Agar

Tryptic Soy Agar of 40 g by weight was suspended in 1 L of demineralized water in Erlenmeyer flasks and the solution was stirred and heated on a hot plate to homogenize them. The solution was then sterilized in a Hiramaya Autoclave Sterilizer for 15 minutes at 121 °C. The solution was allowed to cool down for a few minutes until its temperature drop to 48 °C before pouring it into sterile petri dishes. After that, the media agar was stored in a freezer at 4 °C before conducting a bacteria count.

2.6 Bacteria counting (Spread plate method)

To implement a serial dilution, 1 mL was taken from the sample and put it into test tube containing 9 mL of sterile water. After that, 1 mL of previous dilution was added to 9 mL of sterile dilution test tube which is labeled as sample 1. Then 1 ml from the previous diluted sample is taken and mixed with 9 ml distilled water and these steps were repeated until 10⁻¹⁰ is achieved. The agar plate was stored in an incubator at 35 °C for 24 hours before counting the bacteria in the plate. After one day, the growth of bacteria in a petri dish will be counted. Samples were removed from the incubator and placed in a bacterial count machine.
Bacteria often populate soils in very large numbers ranging from $10^6$ to $10^8$ cells g$^{-1}$ of soil. In this study, the supernatant was diluted with sterile water ($10^6$) in order for the bacteria to be counted accurately. A diluted bacterial culture makes it easier for counting to take place. The dilution six in triplicates was chosen to achieve greater accuracy for the growth enumeration.

3. Results and Discussions

3.1 Concentration of Mercury using EK and EK-Bio

The results in Figure 3 shows the concentration of mercury in parts per billion (ppb) using EK (distilled water at anode and cathode reservoir) and EK-Bio technique at every 3 cm, 6 cm, 9 cm and 12 cm from the bottom of the tank. After 7 days of treatment, all the sampling points using EK and EK-Bio technique reduced to a lower range compared to the initial value. The removal of mercury is attributed to the migration of mercury ions via the electrokinetic process at the end of cathode reservoir. The concentration of soil when using the EK technique after 7 days shows that the initial concentration is 14 ppb and at the all sampling points, it was reduced until it was between 8.3 ppb to 6.7 ppb. Besides that, Rosestolato et. al. [14] stated that metallic mercury is under the effect of the electric field, which acts as a bipolar electrode, assuming an anodic (positive) polarization on the side facing the cathode, and a cathodic (negative) polarization on the side facing the anode of the biasing circuit. Next, the process of dissolution occurred under anodic polarization conditions at the mercury droplet. In summary, iodide ions have the role of complexing the oxidized form of mercury by making it soluble in water and supporting its movement towards the anode due to the applied electric field. On the other hand, the dissolution of metallic mercury does not required the use of solutions enriched with oxidizing agents such as iodine. In addition, according to Acar et. al. [15], the most important processes in the removal of heavy metals are electromigration and electrokinetic.

The lowest concentration of mercury occurs at the near cathode compartment using EK-Bio. The application of isolated bacteria which is Lysinibacillus fusiformis and the electrokinetic technique was effective in reducing the amount of mercury from 23 ppb to 5 ppb at 6 cm from the bottom. EK-Bio shows that the reduction of mercury is based on the migration of bacteria from the anode to the cathode. The concentration at the anode is between 10 ppb to 15 ppb, followed by a concentration at the middle of anode and cathode between 9 ppb to 12 ppb. Towards the end, the concentration reduced between 5 ppb to 7 ppb. From there, it shows that a significant removal has occurred. Mercury concentrations in soil which normally do not exceed 100 ppb are categorized as harmful. From the results obtained, it is shown that by applying EK-Bio, the amounts of mercury removal are up to lower range mercury in soil in all sampling point. Hence it is proven as previous research had successfully removed mercury by using Lysinibacillus fusiformis whereas the other strain, RS-5, its ability to survive at high temperatures and accumulate mercury efficiently in natural environments. Lysinibacillus sp. shows efficient mercury sequestration and biotransformation [10].
3.2 Removal of Mercury using EK and EK-Bio

The EK technique shows significant mercury removal, where the highest removal percentage of mercury removed occurred at the cathode between 51% and 52%. At the anode compartment, the mercury was removed from 44% to 47% for every depth. Meanwhile, the lowest percentage of mercury removal occurs at the middle compartment. However, electrokinetic remediation technique can remove more than 40% of mercury. According to research by Reddy et. al. [8], the removal of mercury from clayey soil using the electrokinetic technique is possible. From their study, distilled water placed at both the anode and the cathode compartment is able to remove approximately 12.5% of mercury. It is proven that electrokinetics helped to eliminate and remove mercury in soil by using distilled water.

Besides that, the application of EK-Bio shows a tremendous reduction of mercury up to 78% at the cathode compartment and particularly the use of L. fusiformis can migrate and remove the mercury as shown in Figure 4. The trends show that the highest percentage of removal occurs at the cathode compartment (approximately up to 78%) follow by the middle compartment (approximately up to 58%) and the anode compartment (approximately up to 52%). This shows that the L. fusiformis migrates with the absence of electric current to the cathode compartment and that L. fusiformis uses mercury to survive [16]. Other researchers also succeed to remove mercury between 80% to 89% by using Pseudomonas putida in wastewater samples [3]. Therefore, using the combination of bacteria and electrokinetic systems, the capabilities of this technique can be improved to remove and reduce the amount of heavy metals [17].
Figure 4. Removal of Mercury a) near the anode, b) near the middle and c) near the cathode using EK and EK-Bio at 3 cm, 6 cm, 9 cm and 12 cm

4. Conclusion
Lysinibacillus fusiformis was effectively used to remove the mercury in the soil with the presence of electric current. From Figure 4, it can be observed that the removal percentage of mercury is highest at the cathode compartment. From the results, using the electrokinetic technique can remove between 40 % to 52 % of mercury but when the technique applied was combined with Lysinibacillus fusiformis, it could remove up to 78 % of mercury. This is supported by Peña-montenegro and Dussán [18] where Lysinibacillus was found to have the potential to remove heavy metals via the bioremediation technique. In summary, this technique has the potential to be highlighted as a form of green technology in the remediation of contaminated soil.

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