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Combination of pseudomonas putida and EK method to reduce the amount of mercury on landfill soil

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Abstract. Landfills usually lack of environment measures especially on soil. There are no guarantee that the landfill soil is free from being contaminated. It may cause harm for humans, animals and plants at surrounding area. In order to solve this problem, advance remediation technique is essential such as the electrokinetic combined with microorganisms known as electrokinetic bioremediation technique. The aim of this study is to investigate the performance of \textit{P.putida} with 15 volt electric current supply (Ek-bio) and without electric current (Bio) in removal of mercury in landfill soil. Both treatments were running throughout 14 days. The \textit{P.putida} was placed at anode compartment meanwhile sterile distilled water poured at cathode compartment. According to the both results, Ek-bio was removed mercury up to 48 % but by using standard bioremediation treatment, the removal only 32 %. Besides that, the migration of \textit{P.putida} react more aggressively during the present of electric current compared with bioremediation. As the results, it was proven that by using Ek-bio technique can increase the activity of bacteria beside and the removal of mercury. Therefore, Ek-bio method can be commercialized to the parties concerned to solve the contaminated soil by mercury.

1. Introduction
Landfill is the most frequent waste disposal method worldwide. Since it is a place for waste disposal, so there may be a various contaminants have been spread around the landfill area [1-3]. Landfill usually lack of environment measures. There is no guarantee that landfills are free from being contaminated especially by heavy metals. Thus, it may cause harm to humans, animals and plants that live around that area. Then, this landfill soil needs to be treated. As we know, there are many technologies have been develop such as thermal, physical/chemical and biological [4-5]. The technique remediation of contaminated soil with electrokinetic will enhance the effectiveness of treatment at the landfill soil [6].

The application of electrokinetics helps to transport the ions where the electric field is applied along the soil, positive ions will be attracted to the cathode and negative ions to the anode. As well as objects such as pollutant molecules or bacteria in the soil will be affected by the process and electrokinetic forces that had been applied [7]. 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 [8,9]. This technology can be applied to the contaminant concentration range less than 100 ppm, but may not be effective for treating multiple contaminants that have significantly different concentration [10].

Other than that, the application of electrokinetic can be combined with microbial (electrokinetic bioremediation) to improve the performance of this method [11-13]. The microbial have a potential to reduce the amount of toxic in soil and can change the contaminants to less hazardous product. There are three different types of in situ bioremediation process which are bioattenuation, biostimulation and bioaugmentation [14]. Bioattenuation is greatly depends on the natural process of degradation while biostimulation is an intentional stimulation of degradation of chemicals is achieved by addition of water, nutrient, electron donors or acceptors. Subsequently followed by bioaugmentation where the microbial members with proven capabilities of degrading or transforming the chemical pollutants are added. In addition, there are several factors that contribute to the suitability of a particular bioremediation technology such as site conditions, indigenous population of microorganism, and the type, quantity and toxicity of pollutant chemical [15]. Besides that, the bacteria was used need to be select based on the capability itself to survive in the contamined soil [4].

Therefore, this study was used *pseudomonas putida* as an agent to reduce the amount of mercury in landfill soil. The selection of this bacteria based on their potential to survive in the contaminated soil that contained harmful compounds [18]. Azoddein et al. [16] used *pseudomonas putida* to removed the mercury up to 80 %. Besides, *pseudomonas putida* combined with electric current also was used to treat the soil contaminated by zinc and lead, and was removed up to 89 % respectively [11]. Based on the previous studies, it was proven that *pseudomonas putida* can be used to treat contaminated soil with existance low voltage of electric current to reduce the amount of mercury in landfill soil that convenient with the aim of this study.

### 2. Materials and Methods

#### 2.1. Location of soil sample

The soil samples were collected at coordinate 2.041317, 102.673008. In this study, standard used according to ASTM D5633-04 (2012) Standard Practice for Sampling with Scoop. The samples were collected from site by pushing the blade of a shovel into the soil to the 10-15 cm depth beneath the surface shown in the figure 1. Then, cut out a triangular wedge of soil by the shovel and set it aside (to be replaced after sampling). After that, push the blades into the soil again taking a thin (1.5 cm) slice from one side of the hole. With a knife, trim the slice to about 2.5 cm strip of soil down the center of the spade-top to bottom. Take the core as part of composite laboratory samples. The core samples were kept in flasks and subsequently transported to laboratory (USDA, 2009). Soil was oven-dried at 105 °C for 24 hours, grinded by a mortar grinder and then sieved to remove plants and debris.

![Figure 1: Scope was pushed into the soil to a depth 10-15 cm.](image)

#### 2.2. Soil sample preparation

Before the treatment was started, there were several step needed are taken. First, approximately 4 kg of soil was mixed with 4 kg of deionized water. The purpose of using deionized water was to prevent all the ions in the soil from attracted to the water before the testing started. Then, the soil sample was
stirred using mechanical machine around 30 minutes and poured into transparent acrylic box. The slurry sample was kept overnight before undergoes consolidation process. Figure 2 shows the consolidation process to compact the soil and to make sure there are no excess water in soil. The consolidation process was running for three stage. First stage with loading 15 kg followed by 30 kg and the last stage is 60 kg. The selection of loading during consolidation was done according to the research by Azhar [17].

![Consolidation process](image)

**Figure 2.** Consolidation process.

2.3. *Preparation of nutrient broth*
Approximately 30 g of soybean casein digest medium broth powder was mixed with 1000 ml in conical flask that had been filled with distilled water. Then, the mixture was mixed slowly until it is well mixed approximately around 45 min. Next, the mixture was placed into the autoclave machine to sterille at 121 °C for 15 min and the mixture solution was cooled down for a few minutes around 35 °C to 37 °C. After the mixture solution has cool down, pour a loop of *pseudomonas putida* into the nutrient broth. Then, it was leave into the incubator shaker at 32 °C and 200 rpm. After 24 to 48 h, the nutrient broth was ready to put in the tank at anode compartment.

2.4. *Preparation of nutrient media*
Approximately 24.2 g of agar powder of pseudomonas agar base mix with 500 ml of distilled water. The mixture was stired until it well mixed. After that, the mixture was placed in the autoclave at 121 °C for 15 min. The mixture was leave in a few minutes until it reach 48 °C then poured into petri dish. Agar media was keep in cooler at 4 °C before it been used for bacteria counting experiment.

2.5. *Preparation of electrokinetic*
Electrokinetic bioremediation tank was design by using transparent acrylic sized 20 cm with diameter of 22 cm as shown in figure 3. Contaminated soil were divided into three portions and paced in the electrokinetic cell. Filter paper and stainless steel plate was placed at the both end of soil. Cathode and anode part are considered as water reservoir. Anode part was filled with bacterial solution where the bacteria act as an agent to treat the contaminated soil. Whereas, distilled water was placed at the anode part. Liquid at both part was fixed at the highest level to prevent the differences in hydraulic gradient. 15 V of direct electric current was applied at the anode and cathode for 14 days. After 14 days, the electric current was stoped and the sample was collected for mercury testing using mercury analyzer model MA-3000 RD/SCC.
3. Results and Discussions

3.1. Removal of mercury by Pseudomonas putida

Both initial concentration of mercury before treatment is 16.615 ppb. Data collection for mercury was obtained based on the distances differences of soil between anode and cathode. Data obtained was classified into five section that was labelled as 3 cm, 6 cm, 9 cm, 12 cm and 15 cm from anode. The reaction between bacteria was resulted the changes in landfill soil in term of differences contamination of mercury. At the same time, from the separation parts of soil, it can be identified whether mercury contaminants at soils has a changes or not. By referring to figure 5 for bioremediation, it was found that the lowest removal of mercury were at the middle section of the soils between anodlyte and cathodlyte (6 cm from anode) which is 7 % removal. Then for section 9 cm, the amount of mercury removal was increase to 32 % ppb. Meanwhile, at section 12 cm and 15 cm, the removal was decreased to 28 % and 26 % respectively. The results showed that the pattern removal of mercury is not sequence and slightly. It is effected because there are no element to transport \textit{P.putida} from anode to cathode. However, it was showed \textit{P.putida} can migrate itself without any supported from other sources. Therefore, it can be concluded that \textit{P.putida} also can reduce the small amount of mercury in soil without using any combination method.

In the other hand, the combination of \textit{P.putida} and electric current can influence in removal of mercury. With low voltage electric current can help the migration of \textit{P.putida} through the soil from anode to the cathode to reduce the amount of mercury [12]. From figure 5 for Ek-bio, it shows that the trend removal is same with bioremediation but the removal of mercury is highest than bioremediation.
The lowest removal was occurred at 6 cm which is 33%. The removal was increase at 9 cm to 48% and continue decrease at 12 cm and 15 cm. Both techniques shown the same pattern where the lowest removal was occurred at 6 cm. While, the highest removal was occurred at 9 cm for bioremediation while 12 cm for Ek-bio. The lowest removal for both treatment was occurred at 6 cm caused by the activities of \textit{P.putida} during the treatment. When the treatment has been stopped then caused the activities of \textit{P.putida} was interrupted and cause the removal is low. Based on this result, it can be conclude with the addition of electric current through the soil can increase the removal of mercury [13]. So, it was proven that Ek-bio was more effective compared to bioremediation.

![Figure 5](image)

**Figure 5.** Concentration of mercury after Ek- bio and bioremediation treatment for 14 days.

3.2. Migration of \textit{Pseudomonas putida} through soil

The migration of \textit{P.putida} was recorded at three stages. First, the data was recorded before running the testing and was recorded as NB before treatment. Second, after 14 days treatment (NB after treatment) and lastly at the five different location of soil sample after treatment. Before that, the bacteria was count for soil before treatment to measure the amount of \textit{P.putida} and the results is zero which there are no \textit{P.putida} colonie was appear during counting. For the initial stage, the amount of nutrient broth \textit{P.putida} was recorded. As shown in figure 6, both pattern show the reduction amount of \textit{P.putida} occurred after treatment 14 days. Although, the pattern migration of \textit{P.putida} from anode to cathode through soil was different for Ek-bio and bioremediation. The pattern migration for bioremediation is decreasing from 3 cm to 15 cm which are 42 x 10^5 cfu/g, 31 x 10^5 cfu/g, 31 x 10^5 cfu/g, 24 x 10^5 cfu/g and 23 x 10^5 cfu/g, respectively. Then, the migration of \textit{P.putida} for Ek-bio is increasing where the amount of \textit{P.putida} is always increase start from 3 cm (104 x 10^5 cfu/g), 6 cm (136 x 10^5 cfu/g), 9 cm (143 x 10^5 cfu/g), 12 cm (145 x 10^5 cfu/g) and 15 cm (152 x 10^5 cfu/g). Therefore, the result migration of \textit{P.putida} was supported by Deflaun and Condee [12] which is by using low voltage of electric current can increase the migration of \textit{P.putida} in soil and also can increase the amount of \textit{P.putida} [17-18].
4. Conclusion
The combination of P.putida and electrokinetic method used to reduce the amount of mercury on landfill soil was successful and the method of research was presented. The P.putida and with the presence of low voltage of electric current do not give negative impact to lifespan of P.putida. By comparing the both method using only P.putida and P.putida with electrokinetic method on landfill soil, the combination technique give more improvement in removal of mercury. The results give meaningful contribution to the environmental engineers and geotechnical engineers in solving the problem of mercury in soil. In addition, this method is the most recommended technology where it is also can be categorized as green remediation technology.

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