Evaluation of Phytoremediation and Bioremediation for Sandy Soil Contaminated with Petroleum Hydrocarbons

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***Abstract*—Soil contamination with petroleum hydrocarbons causes many problems for the surrounding environment. The current research aims at studying the performance of different in-situ remediation methods for the removal of total petroleum hydrocarbons (TPH) from sandy soil at different levels of contamination. The remediation methods that were tested include phytoremediation using alfalfa, bioremediation using Pseudomonase putida, and a combination. The soil was spiked with TPH at different levels (2.5%, 5.0% and 10.0%). after 90 days of experiments, the different treatments were able to reduce the level of contamination in the sandy soil with efficiencies up to a maximum of 99.9% for phytoremediation, 98.7% for bioremediation, and 99.0% for the combination method. The experimental results showed that the TPH remediation followed the first-order kinetics.**

***Index Terms*—Bioremediation, phytoremediation, contamination, hydrocarbon.**

I. Introduction

Contamination of soil with petroleum hydrocarbons became a global problem due to the hazardous effects on the surrounding environment. Soil contamination with petroleum hydrocarbons takes place from many sources such as production, transportation pipelines and tankers, refineries, storage tanks, and accidents. It is now widely recognized that soil contamination with petroleum hydrocarbons is a potential threat to human health that forced the efforts in the last centuries to find efficient, low cost and environment friendly techniques for soil remediation such as Phytoremediation and bioremediation [1], [2].

Petroleum hydrocarbons degradation by Plants and microorganisms take place directly and indirectly by converting petroleum hydrocarbons into products (e.g., alcohols, acids, carbon dioxide, and water) that are generally less toxic and less persistent in the environment [3]. Phytoremediation of soils contaminated with various hydrocarbons using hybrid poplar tree can reduce concentrations of benzene, toluene, ethylbenzene, xylene, and gasoline range organics to an average of 81%, 90%, 67%, 78%, and 82%, respectively [1].

Bacteria are used for biodegradation of soil pollutants by removing or detoxifying that is called bioremediation technology [4]. *Pseudomonas pseudoalcaligenes*, *Bacillus firmus*, *Bacillus alvei*, *Penicillium funiculosum*, *Aspergillus sydowii* and *Rhizopus sp.* can removed 79%, 80%, 68%, 86%, 81% and 67% of TPH, respectively in 15 days experiment [5].

Very high TPH concentrations inhibit both normal germination of plant seeds and microbial activities in the contaminated soil due to the toxicity of TPH to the plant and bacteria [4]-[6]. In all contaminated cultivated soils, the reduction of crude oil was higher than non-cultivated soils, except 10% contamination level that inhibit the germination and normal growth of alfalfa [7]. Increasing of contamination levels of benzene in soil, will generally increase the duration needed for bioremediation [8]. Using phytoremediation and bioremediation methods for contaminated soil enhance the physical and chemical characteristics of the soil [2], [6], [9]. Plant and bacteria can independently remove hydrocarbon from contaminated soil [9].

This work aims at evaluating the use of alfalfa, *Medicago sativa L.*, bacteria, *Pseudomonase putida*, or a combination of both in the remediation of sandy soil contaminated with TPH.

II. Materials and Methods

A. Experimental Conditions

All experiments were carried out in a field environment at a temperature range from 5 ^{o}C to 40 ^{o}C and an average relative humidity of 76%. The indicated experimental conditions support the growth of bacteria and plants used in the study.

B. Experimental Set-up

All experiments were carried out in plastic pots. Each pot is cylindrical in shape with a diameter of 12 cm and a height of 11 cm. Each pot was filled with approximately 800 g of soil. To study the remediation of soil at different contamination levels, the soil was spiked with three different levels of TPH contamination. Each level of contamination was subjected to four types of treatments. In the first type, Treatment 1, bacteria were added to the contaminated soil to investigate their ability in the removal of TPH from soil. In the second type, Treatment 2, plants were grown in the contaminated soil to study their ability for soil remediation. In the third type, Treatment 3, the synergic effect of using plants and the bacterial culture were investigated on the removal of TPH from the contaminated soil. The last type of treatment, Treatment 4, is the control. Control involved the use of soil without the addition of bacteria or growing plants to

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investigate the no action conditions. A blank pot (no spiking and no treatment) was also tested to determine any background contamination in the soil before spiking and any contamination that might take placed during the experimental period. In addition, alfalfa was grown in clean soil (no spiking with TPH) to monitor the plant growth in the un-contaminated soil conditions.

C. Soil Used in the Experiments

The soil used in the experiments was sandy soil. The soil was then spiked with petroleum hydrocarbons to reach different levels of soil contamination with TPH. These levels include 2.5%, 5.0% and 10.0%. The soil was spiked with a solution contains TPH with 62% in the gasoline range organics (C-6 to C-12), and 27% in the diesel range organics (C-13 to C-28).

D. Plant Used in the Experiments

Alfalfa plant, *Medicago sativa L.*, was used in Treatments 2 and 3. In these two treatments, about 3g of alfalfa seeds were spread on the soil surface of each pot. The seeds started to germinate from the second day after spreading on the soil surface. The percentage of seed germination was about 90%. The pots containing plants were watered daily by spraying 30 ml of water.

E. Bacteria Used in the Experiments

The bacterial culture was prepared by isolating the selected strain of aerobic bacteria from a sandy soil sample collected from a site historical contaminated with TPH. The soil sample was collected from a location near a tank that stores petroleum product with the same TPH structure used in this experiment. Bacteria were isolated from the contaminated soil by molecular characterization method [10]. The isolated strain of bacteria includes *Pseudomonase putida*. A solution that contains the isolated strains was prepared with a bacterial culture concentration of 10^8 CFU per gram of solution. The soils in Treatments 1 and 3 were spiked with 100 mL of the bacterial solution.

F. Sample Analyses

To evaluate the performance of each remediation technique, soil samples were collected from pots throughout the experiment after 15, 30, 60, and 90 days. This is in addition to the samples collected at the beginning of the experiments. Samples were analyzed for TPH according to U.S. EPA, 1998 (Method 9071B) [11]. At the indicated times, pots of different treatments at different levels of contamination were taken out from the experimental set up. Then, the soil of each pot was well mixed before sampling. About 5 g of well mixed soil were sampled for TPH analysis. Each sample was subjected to continuous washing with 90 ml of hexane through Soxhlet apparatus to extract the TPH from the soil sample. About 3 ml of the extract was added to a vial with a Teflon cap for injection into a Gas Chromatography (Hewlett Packard GC, model 5890, series equipped with flame ionization detector).

III. Results and Discussion

Experiments were conducted to investigate the remediation of soil at different contamination levels during 90 days. The remediation includes four different treatments as indicated before. The soil was analyzed throughout the experiments to evaluate the effect of different treatments on the TPH removal as well as determining the removal rate kinetics. Fig. 1 shows the change of TPH concentration with time in soil spiked with TPH at 2.5% for different treatments. Fig. 2 shows the change of TPH concentration with time in soil spiked with TPH at 5% for different treatments. Fig. 3 shows the change of TPH concentration with time in soil spiked with TPH at 10% for different treatments.

A. Evaluation of the Different Remediation Methods

In Treatment 1, the highest removal efficiency of TPH was obtained after 90 days at all contamination levels. The highest removal efficiencies using this treatment ranged between 96.7% at the 5% contamination level and 98.7% at the 10% contamination level. Similar effect of bacterial culture in 5% diesel contaminated soils was reported; hence the observations recorded complete disappearance of n-C12-20 and 75% reduction of TPH after 42 days of bioremediation [12].

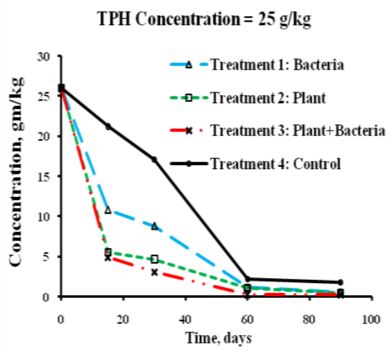

Fig. 1. TPH concentration during remediation process of hydrocarbon contaminated soils with initial contamination 2.5%.

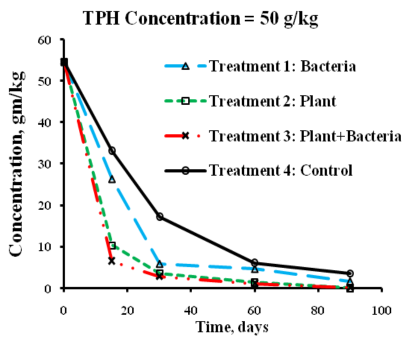

Fig. 2. TPH concentration during remediation process of hydrocarbon contaminated soils with initial contamination 5%.

Similar trends were observed with other treatments. However, in Treatment 2, the removal efficiencies after 90 days ranged between 97.7% at the 10% contamination level and 99.9% at the 5% contamination level. In Treatment 3, the synergic effect of bacteria on the removal of TPH had no significant effect on the removal efficiencies by plants. In this treatment, after 90 days, the TPH removals ranged between 98.3% at the 10% contamination level and 99.7% at the 5% contamination level.

Significant reduction of TPH contamination has been observed in control samples after the experiment period due to the environmental conditions and native microorganisms in the soil. Such significant reductions take place due to the use of light hydrocarbons fractions as a contaminant that have high volatility and high water solubility, this approach agreed with the findings of Alisi *et al*. (2009) [12] who found that the concentration of C12 decreased in the control sample by 65% at the end of 24 days experiment due to environmental conditions. Also Collins (2006) [13] mentioned that decreasing carbon chain length of the hydrocarbon contaminant, will increase its solubility and decrease its partition to soil, and hence increase contaminated soil remediation efficiency.

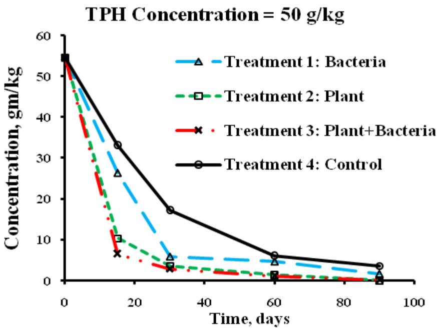

Fig. 3. TPH concentration during remediation process of hydrocarbon contaminated soils with initial contamination 10%.

In general, the TPH concentration decreased with time even with controls. Fig. 1 to 3 shows that the use of Treatment 1 (bacteria) can improve the removal of TPH from the contaminated soil. This is concluded from the significant decrease in TPH concentration in soil with Treatment 1 as compared to that of Treatment 4 (control). Fig. 1 to 3 also shows that Treatment 2 (plant only) and Treatment 3 (plant + bacteria) significantly improve the remediation of soil as compared to Treatments 1 and 4.

In general, the TPH concentration in soil decreased significantly within the first 15 days of the experiment with the use of Treatments 2 or 3 for all levels of contamination. This is compared to 30 - 60 days with other treatments. After 60 days, the changes in the remaining concentration of TPH in soil became small or insignificant. As shown in the Fig. 1 to 3, the remediation efficiencies of different treatments follow the following order in the removal of TPH; plant + bacteria ≥ plant only > bacteria > control.

However, as shown in figures, the difference between the performance with the use of Treatment 3 and Treatment 2 is insignificant. Therefore, phytoremediation using plant only (Treatment 2) can be considered as the most suitable technique to be used for the removal of TPH from sandy soil. With the use of Treatment 2, the TPH concentration in soil decreased by about 78.6%, 80.9%, and 88.7% within the first 15 days at initial TPH contamination levels of 2.5%, 5.0% and 10.0% respectively.

B. Kinetics of TPH Removal

The kinetics of TPH removal using plants, bacteria and a combination was investigated. It was found that the removal of TPH in all treatments followed a first order removal kinetics. Equation 1 ahows the kinetic equation that represent the removal of TPH from soil using any of the treatments.

$$C/C_o = e^{-kt} \quad (1)$$

where C is the TPH concentration at time t, C_o is the initial TPH concentration at $t = 0$, and k is the removal rate constant.

Results showed that the highest reaction rate constant (k) was observed with Treatments 2 and 3 and its value was 0.059 day^{-1} in both treatments.

C. Mechanism of TPH Removal

Plants and bacteria can degrade hydrocarbons contaminants from the soil directly or indirectly. Direct processes include contaminants uptake and transformation, storage, or transpiration, while indirect process is the degradation of contaminants by plant roots exudates and microbial activities [14]. In the recommended treatment, Treatment 2, alfalfa roots penetrate the soil allowing oxygen concentration to increase in soil rhizosphere. This enhances the microbial activities and population, which improves the degradation of hydrocarbons in the contaminated soil [12]. Also alfalfa increased the efficiency of hydrocarbons degradation due to the effect of root exudates such as carbon, nutrients and enzymes that enhance microbial populations in the rhizosphere [15]. In Treatment 1 (bacteria), the reduction of TPH was suggested to be due to the fact that bacteria take the hydrocarbon contaminants as a supplement for population. Bacteria take the hydrocarbon contaminants as source of carbon for new cells constituents and bacteria release enzymes in the soil, which have the ability to catalyze the oxidation of hydrocarbons contaminants [2]-[16].

IV. Conclusion

Experiments were conducted to investigate the ability of different treatments in the remediation of sandy soil contaminated with different levels of TPH. Results showed that, for all treatments, the highest rate of TPH removal was during the first 15 days from the start of the remediation. After that, the removal process of TPH became slower up to day 60. Then, no significant difference was observed for TPH removal till the end of the experiments. Results also showed that there is no significant difference in the performance of phytoremediation using plant only and using Plant-bacteria combination in the removal of TPH. The remediation efficiencies of different treatments follow the following order in the removal of TPH; alfalfa plant + *Pseudomonase putida* bacteria ≥ alfalfa plant only > *Pseudomonase putida* bacteria > control.

Overall, Phytoremediation using alfalfa is recommended for use in remediation of sandy soil contaminated with TPH with levels up to 10%. With the application of phytoremediation, most of TPH is removed within the first 15 days from the start of the remediation. After 60 days, the alfalfa will have a very small effect on the removal of TPH from the contaminated soil. In addition, the kinetics study of the data obtained from the experiments showed that TPH removal followed first-order kinetics with a rate constant k of 0.059 day^{-1}. After 90 days of remediation, the removal of TPH can reach up to 99.9% when phytoremediation with alfalfa is employed.

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