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Bioremediation of Contaminated Clay Soils

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A B S T R A C T

This research work demonstrates the feasibility of accelerating bioremediation of a clay soil by supplementing with $(NH_4)_2SO_4$, KH_2PO_4, sucrose (as an inducer for growth) and tween 80. The soil contained 7% residual gasoil. The bioremediation was stimulated by moisture adjustment to 10%, and inoculating with hydrocarbon degrading microorganisms. $(NH_4)_2SO_4$ and KH_2PO_4 were added to the soil to obtain soil samples with C:N:P ratios of 100:1.4:1.4, 100:6.4:1.9, 100:11.4:2.4, and 100:21.4:3.4. The C:N:P of 100:11.4:2.4 resulted in more than 78% gasoil removal for duration of two months. Tween 80, in the range of 0-20mL/ (kg soil), was added to the soil samples with C:N:P ratio of 100:1.4:1.4. More than 84% removal was achieved when 10 mL/kg tween 80 was added to the soil. Sucrose, in the range of 0-20 g/(kg soil), was added to the soil samples with the C:N:P ratio of 100:1.4:1.4. For the sucrose level of 20 g/(kg soil), 79% removal was obtained in two months. Additional experiment was also conducted at two porosity levels of 54% and 22%. The removal percentage in the soil with high porosity was almost twice as compared to soil with low porosity.

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INTRODUCTION

Hydrocarbon contaminations in soil are one of the most important environmental issues in oil rich countries. Soil contamination with hydrocarbons may occur during oil exploration, refining, and transportation [1]. The presence of hydrocarbons have adverse effects on the activity of living organisms in soil [2].

Bioremediation is a process that uses microorganisms to clean up contaminated soils. Various factors influence the rate of the process. Moisture, soil type, porosity, availability of nutrients, and microbial types are among the factors that affect bioremediation process.

Lack of nutrients, particularly nitrogen and phosphorus, is the most common limiting factor in the process of bioremediation[3].Nutrient requirement depends on the origin and the nature of the contaminated soil.The nutrient content of contaminated soil should be examined before bioremediation, and nutrients should be added if necessary. Nutrient addition should be done with care since excessive concentration of nutrients can have inhibitory effects on the activity of degrading microorganisms. Nutrients can be dissolved in water and then added to the soil as fertilizers [4]. Addition of low levels of an easily degradable carbon source (such as sucrose) may also enhance soil bioremediation by rapid proliferation of microorganisms at the beginning of the process.

The use of surfactants in the bioremediation of contaminated soils is one of the strategies to increase the mobilization of hydrophobic organic contaminants in soil [5]. Surfactants can increase the bioavailability of contaminants for degrading microorganisms in the soil [6-8]. Surfactants can decrease interface tension and increase the apparent aqueous solubility of hydrophobic compounds, thus facilitating mass transfer of these materials from solid into aqueous phase. Surfactants may also strengthen cell surface hydrophobicity, which leads to rapid hydrocarbon utilization [9-10]. However, surfactants are not always beneficial to bioremediation. In general surfactants may promote or inhibit hydrocarbon biodegradation [11-12]. Drastic change in cell surface hydrophobicity, high concentrations, and being a preferred carbon source are some reasons for negative impact of surfactants on bioremediation [13].

Another factor affecting the rate of bioremediation of soil is porosity. Bioremediation is influenced by the grain structure and the space between the grains in the soil. Akbari et al. [14] found that microorganisms do not grow in pore spaces less than 3 micrometer. They also found that the microstructure of the soil can affect the removal rate in bioremediation of soil.

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In this research work bioremediation of a highly contaminated clay soil was examined. Bioremediation of clays is challenging due to strong adsorption of organic pollutants to their surface [8,15]. Addition of nitrogen and phosphorous sources, low levels of sucrose, and low levels of a surfactant were considered for improvement of bioremediation. The effect of porosity of the soil was also investigated in this research work.

MATERIAL AND METHODS

Soil

A sample of soil containing 95% clay was used in this work. The soil was categorized based on the Unified Soil Classification System (USCS). An investigation on the origin of the soils indicated negligible organic content. The initially clean soil was contaminated with gasoil (see Table 1) to the concentration of 100 g/kg, and left on the floor at room temperature. After evaporation of the light hydrocarbons, the residual gasoil in the soils was quantified (7%).This soil sample was subjected to bioremediation.

TABLE 1. Gasoil components

Water and Sediments	Ash	Total sulfur	Mass	Compound
0.05%Vol	0.01%Wt	1%Wt	840 kg/m3	C_6-C_{16}

Quantification of total petroleum hydrocarbons (TPH) in soil

The TPH content of the soil samples were extracted by Soxhlet apparatus, and quantified based on EPA Method 9071B.

Effect of nutrient supplementation on the bioremediation of clay soil

$(NH_4)_2SO_4$ and KH_2PO_4 were used as the sources of nitrogen and phosphorous for microorganisms. Four samples with C: N: P ratios of 100:1.4:1.4, 100:6.4:1.9, 100:11.4:2.4, 100:21.4:3.4 were prepared. The weight of each sample was 500 g. Samples were inoculated with 10 mL of a microbial solution (containing hydrocarbon degraders), and placed in PVC columns. The moisture content of the samples was adjusted to 10% with tap water. The columns were kept under ambient conditions in the temperature range of 25-30 °C. The columns were weighed every 48 hours and the loss of water was compensated by tap water. The samples were blended thoroughly after the addition of tap water. The process was monitored for two months. The residual TPH in the columns were determined at the end of the process.

Effect of Tween 80 on the bioremediation of clay soil

Soil samples were supplemented with Tween 80 (Merck) at four levels of 0, 5, 10, and 20 mL/kg.The samples were subjected to bioremediation with microbial inoculation and moisture adjustment. The sample size, columns, duration of the process, initial moisture content, compensation of water loss, and the process condition were exactly the same as explained in previous part. The selected C: N: P ratio of the soil was100:1.4:1.4.

Effect of sucrose on the bioremediation of clay soil

Soil samples (with initial C: N: P ratio of 100:1.4:1.4) were supplemented with sucrose (food grade) at four levels of 0, 5, 10, and 20 g/kg. The process started with microbial inoculation and moisture adjustment. The sample size, columns, duration of the process, initial moisture content, compensation of water loss, and the process condition were exactly the same as explained.

Bioremediation of the clay soil as a function of porosity

The experiment for the investigation on the effect of porosity on bioremediation was designed in two levels. For a high porosity sample, 600 g of the soil sample (with C: N: P ratio of 100:1.4:1.4) was packed in a column with the volume of 850 cm^3. For a low porosity sample, the same volume was packed with 1200 g of the same soil. The porosity of the samples was estimated to be 54 % and 22%. The samples were subjected to bioremediation with microbial inoculation and moisture adjustment (10%). The columns were weighed every 48 hours and the loss of water was compensated with tap water. To keep the porosity constant, no blending of the samples was performed during this experiment. The process was continued for two months. The loss of water was compensated every 48 hours.

Bioremediation of clay soil as a function of time

Bioremediation of the clay soil as a function time was investigated in a separate experiment. Four columns were filled,each with 500 g of the polluted soil. The initial C: N: P ratio for the samples was 100:1.4:1.4. The first column received only microbial inoculation. The second column inoculation, and supplemented with $(NH_4)_2SO_4$ and KH_2PO_4 to C: N: P ratio of 100:11.4:2.4. The third column received microbial inoculation and Tween 80 (10 mL/kg), and finally the fourth column received microbial inoculation, and supplemented with $(NH_4)_2SO_4$ and KH_2PO_4 to C: N: P ratio of 100:11.4:2.4, and Tween 80 (10mL/kg). The conditions of bioremediation were exactly the same as previously described. Samples were withdrawn from the columns in 10 days intervals for the quantification of the residual TPH in soil. The process continued for 60 days.

RESULTS AND DISCUSSION

Effect of nutrient supplements on the bioremediation of clay soil

Figure 1 shows the results of bioremediation with different ratios of C: N: P in the soil. Bioremediation of the sample with C: N: P ratio of 100:1.4:1.4 resulted in 52% TPH removal for duration of two months. Addition of nutrients to the C: N: P ratio of 100:6.4:1.9 improved the removal percentage of TPH to 67%. The removal percentage reached the optimum value of 78% with the C: N: P ratio of 100:11.4:2.4. Higher amounts of the nutrients had a negative impact on the bioremediation; that was probably due to excessive osmotic pressure. Analysis of variance confirmed that nutrient supplementation had a significant effect on bioremediation (Table 2). Similar results were reported by Lee et al [16]. The researchers examined the effect of nutrients on the bioremediation of a contaminated soil with the initial TPH content of 9320 mg/kg. Three C:N:P ratios of 500:10:1, 200:10:1, and 100:10:1 were examined. The best results were obtained for the soil with C:N:P ration 100:10:1 with around 50% TPH removal in 105 days.The removal in the control sample was only 18%.

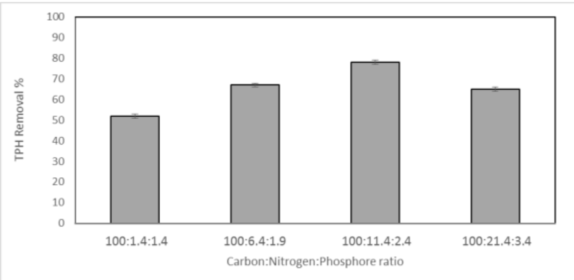

Figure 1. The effect of nutrient supplementation on clay soil bioremediation. Initial TPH: 70 g/kg Process time: 60 days

TABLE 2. Analysis of variance for the effect of nutrient supplementation on the bioremediation of clay soil

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F	P_{value}
Nutrient	3	666.538	222.179	764.97	0.000
Error	4	1.162	0.290		
Total	7	667.700			

Effect of sucrose on hydrocarbon removal

Figure 2 shows the effect of sucrose on the bioremediation of the clay soil. The results indicate that sucrose as a readily accessible substrate has a significant positive effect on the bioremediation. Table 3 summarized the effect of sucrose on the bioremediation is statistically significant. Addition of 20 g/kg sucrose to the soil increased the TPH removal from 50 to 79%. Most soil microorganisms can assimilate sucrose. This substrate is soluble in water and microorganisms can easily uptake contaminants. This can lead to rapid proliferation of microorganisms during early stages of bioremediation. Upon exhaustion of sucrose, the microorganisms degrade hydrocarbons as the source of carbon and energy. Hesnawi et al [17] reported considerable improvement in soil bioremediation by the addition of urea as a nutrient. Around 80% removal was observed when urea was applied to the soil compared to 52% in the control soil. Urea, containing carbon and nitrogen, is a favorable nutrient for most microorganisms in soil. Many waste materials from agriculture or food processing plants contain readily accessible substrates which can be used in soil bioremediation. Overall, it was concluded that readily accessible organic compounds do not repress bioremediation of hydrocarbons, and actually as it was confirmed in this research they can accelerate the process.

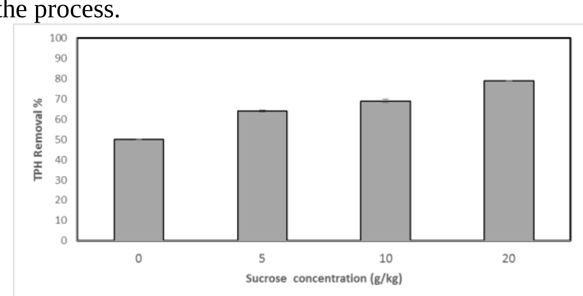

Figure 2. The effect of sucrose supplementation on clay soil bioremediation.Initial TPH: 70 g/kg .Process time: 60 days

TABLE 3. Analysis of variance for the effect of sucrose supplementation on the bioremediation of clay soil

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F	P_{value}
Sugar	3	842.028	280.676	940.54	0.000
Error	4	1.194	0.298		
Total	7	843.221			

Effect of Tween 80 on hydrocarbon removal

Figure 3 shows that Tween 80 affects soil bioremediation significantly. Addition of 10 mL/kg of Tween to the soil resulted in 84% removal of TPH form the soil. The removal percentage was only 50% in the blank sample. Furthermore increase in the dosage of Tween 80 is beneficial up to a certain level, above which the toxicity of the surfactant prevails. The results of analysis of variance for the effect of Tween 80 on the bioremediation of the clay soil are shown in Table 4. Bioremediation of TPH contaminated soil is limited by poor water solubility, and strong adsorption of TPH to soil particles. Increasing TPH solubility in soil aqueous systems can improve mass transfer and bioavailability of the compounds, resulting in enhanced biodegradation. Surfactants can improve hydrocarbon utilization through emulsifications as well as adhesion to microbial cell surfaces [18]. Kim et al. [19] examined several non-ionic surfactants including Tween 80 to enhance bioremediation of hydrocarbons in aqueous and slurry systems. They concluded that Tween 80 caused a significant improvement in the biodegradation of hydrocarbons in aqueous and slurry systems. The results here confirm that Tween 80 also improves bioremediation of wet clay soils.

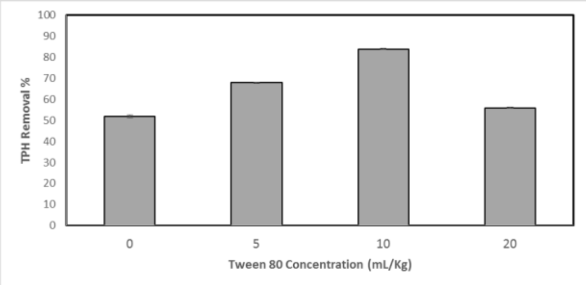

Figure 3. The effect of Tween 80 on clay soil bioremediation.Initial TPH: 70 g/kg. Process time: 60 days

TABLE 4. Analysis of variance for the effect of Tween 80on the bioremediation of clay soil

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F	P_{value}
Tween 80	3	1264.636	421.545	1991.88	0.000
Error	4	0.847	0.212		
Total	7	1265.482			

Bioremediation of the clay soil as a function of porosity

The removal rate in the soil with higher porosity (54%) was significantly greater than the removal rate in the soil with lower porosity (22%). Higher porosity allows diffusion of air into soil matrix and this enhances oxygen transfer rate to the soil. Oxygen is the most widely used electron receptor in bioremediation and its concentration in soil is influenced by soil porosity which is a function of soil type and texture [20]. Higher porosity moreover provides sufficient space for microorganisms to proliferate. Table 5 summarizes the results of analysis of variance for the effect of porosity on bioremediation.

TABLE 5. Analysis of variance for the effect of porosity on the bioremediation of clay soil

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F	P_{value}
Porosity	1	959.9329	959.9329.545	14206.98.88	0.000
Error	2	0.1351	0.0676		
Total	3	960.0680			

Bioremediation of clay soil as a function of time

Figure 4 shows the TPH removal in clay soil as a function of time. The bioremediation rate was relatively slow when there was limited nutrient supplement. The removal percentage reached to 51.5% in 60 days. The process accelerated to some extent when the soil was supplemented with nitrogen and phosphorus sources. In this case the removal percentage of 71% was obtained in 60 days. Addition of Tween 80 had a positive effect on the bioremediation rate. Addition of 10 mL/kg of Tween 80 to the soil increased the TPH removal percentage to 81.5% in 60 days. The maximum bioremediation rate was obtained when the soil was supplemented with both nutrients and Tween 80. In this case the removal percentage approached to 90% in 50 days. The results confirm that bioremediation occurs with moisture adjustment and intermittent mixing, but the rate of bioremediation can be improved considerably with nutrient and/or surfactant supplements.

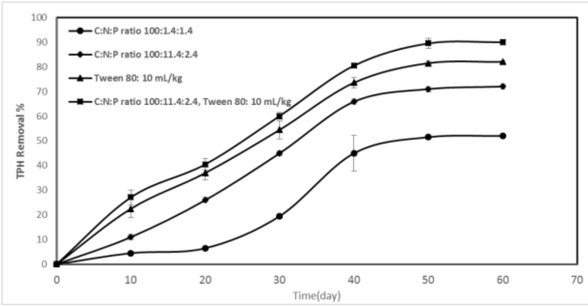

Figure 4. Bioremediation of clay soil as a function of time. Initial TPH: 70 g/kg.

CONCLUSION

Bioremediation of clay soil contaminated with high levels of TPH is possible. Frequent mixing and moisture adjustment are key parameters in clay soil bioremediation. The rate of bioremediation can be improved considerably by supplementation of the soil with nitrogen and phosphorus sources. The ratio of C: N: Pequal to 100:11.4:2.4 was found in this research as the optimum value for bioremediation of clay soils. Addition of sucrose, as a readily accessible substrate, is useful in improving the rate of bioremediation. Tween 80 as a non-ionic surfactant would have a positive effect on bioremediation of clay soil if a suitable dosage is used. Nutrient supplement together with additional surfactant was found in this research as the most efficient method for the bioremediation of clay soil.

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Persian Abstract

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چکیده

این کار تحقیقاتی امکان سنجی تسریع بهبود زیست خاک خاک را با افزودن NH4) 2SO4)، KH2PO4، ساکارز (به عنوان القاء رشد) و tween 80 نشان می دهد. خاک شامل ۷ درصد گازوئیل باقی مانده است. تصفیه بیولوژیک توسط تنظیم رطوبت به ۱۰ درصد تحریک شد و با میکروارگانیسم های تخریب شده با هیدروکربن تلقیح شد. NH4) 2SO4) و KH2PO4 به منظور تهیه نمونه های خاک با مقادیر C: N: P 100: 1.4: 1.4، 100: 6.4: 1.9، 100: 11.4: 2.4، و ۱۰۰: ۲۱٫۴: ۳٫۴، به خاک افزوده شد. C: N: P از ۱۰۰: ۱۱٫۴: ۲٫۴ باعث حذف بیش از ۷۸٪ گازوئیل در مدت دو ماه شد. Tween 80 در محدوده ۰-۲۰mL / (کیلوگرم خاک) به نمونه های خاک با نسبت C: N: P به ۱۰۰: ۱٫۴: ۱٫۴ اضافه شد. بیش از ۸۴٪ حذف زمانی که ۱۰ میلی لیتر / کیلوگرم بین ۸۰ به خاک اضافه شد. ساکارز در محدوده ۰-۲۰ گرم در کیلوگرم به نمونه های خاک با نسبت C: N: P به ۱۰۰: ۱٫۴: ۱٫۴ اضافه شد. برای میزان ساکارز ۲۰ گرم / (کیلوگرم خاک)، حذف ۷۹ درصد در دو ماه به دست آمد. آزمایش های دیگر نیز در دو سطح تخلخل ۵۴٪ و ۲۲٪ انجام شد. درصد حذف در خاک با تخلخل بالا تقریبا دو برابر نسبت به خاک با تخلخل پایین بود.