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Research article

The effect of soil type on the bioremediation of petroleum contaminated soils

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ABSTRACT

In this research the bioremediation of four different types of contaminated soils was monitored as a function of time and moisture content. The soils were categorized as sandy soil containing 100% sand (type I), clay soil containing more than 95% clay (type II), coarse grained soil containing 68% gravel and 32% sand (type III), and coarse grained with high clay content containing 40% gravel, 20% sand, and 40% clay (type IV). The initially clean soils were contaminated with gasoil to the concentration of 100 g/kg, and left on the floor for the evaporation of light hydrocarbons. A full factorial experimental design with soil type (four levels), and moisture content (10 and 20%) as the factors was employed. The soils were inoculated with petroleum degrading microorganisms. Soil samples were taken on days 90, 180, and 270, and the residual total petroleum hydrocarbon (TPH) was extracted using soxhlet apparatus. The moisture content of the soils was kept almost constant during the process by intermittent addition of water. The results showed that the efficiency of bioremediation was affected significantly by the soil type ($P_{value} < 0.05$). The removal percentage was the highest (70%) for the sandy soil with the initial TPH content of 69.62 g/kg, and the lowest for the clay soil (23.5%) with the initial TPH content of 69.70 g/kg. The effect of moisture content on bioremediation was not statistically significant for the investigated levels. The removal percentage in the clay soil was improved to 57% (within a month) in a separate experiment by more frequent mixing of the soil, indicating low availability of oxygen as a reason for low degradation of hydrocarbons in the clay soil.

1. Introduction

Petroleum hydrocarbons are persistent pollutants in the environment. Uncontrolled release of these compounds affects soil, water, and air negatively (Ulrici, 2008). Soil pollution with hydrocarbons is caused by the leakage from underground reservoirs, petroleum refineries, storage facilities, and accidental spillage from production units and transport pipelines. The presence of petroleum hydrocarbons affects physical, physiological and biochemical properties of soil (Margesin et al., 2003; Head et al., 2006). Plants are susceptible to oil exposure due to phytotoxic nature of hydrocarbons, and immobilization of nutrients in the soil (De Jong, 1980; Udo and Fayemi, 1995). Inherent mutagenic properties of some hydrocarbons and their low degradation rates require special attention to remediate these pollutants (Johnsen et al., 2007).

Various physical, chemical and thermal methods have been employed to clean up oil contaminated sites (Frick et al., 1999). More recently, bioremediation has been suggested as a cost effective and environmental friendly method for soil cleaning. In bioremediation the capability of biological agents is exploited to degrade hydrocarbons. A large body of literature exist that suggest bioremediation as a cost effective and environmental friendly method of soil cleaning (Kwok and Loh, 2003; Glick, 2003; Zhuang et al., 2007; Gerhardt et al., 2009; Dindar et al., 2013; Sarma Roy et al., 2014).

Moisture content of soil, microbial population, nutrient availability, soil type, salinity, and oxygen transport in soil are among the factors affecting the process of bioremediation. The moisture content of soil should be at an optimum range. Low levels of moisture content decrease microbial activity, while excess water may create resistance to oxygen transfer and may also produce an unwanted leachate (Schjønning et al., 2011). In bioremediation the

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Table 1
Bandar Abbas gasoil components.

Water and sediments	Ash	Total sulfur	Density	Compound
0.05%Vol	0.01%wt	1%wt	840 kg/m^3	C_6–C_{16}

moisture content is normally adjusted to a fraction of water holding capacity of the soil. The optimum value of moisture, however, is a function of soil type, pore size distribution, and soil texture. The information in the literature on the optimum value of the moisture content of soil for the purpose of bioremediation is scarce, and more investigation is needed to address this case. In general, optimum microbial activity is achieved by the maximum water content that does not restrict oxygen diffusion. Nitrogen, phosphorous, sulfur and some other nutrients are necessary for microbial growth and activity. Therefore contaminated soils should contain ample amounts of these elements for a successful bioremediation process. As a rule of thumb the ratio of Carbon: Nitrogen: phosphorous should be 100:10:1 to ensure a balanced medium for microbial growth in term of nutrients (Prescott et al., 2002). Soil type and texture can affect bioremediation. Fine grained soils like clay have low permeability and retard oxygen and nutrients transport in the soil. Controlling the moisture content in fine grained soils is difficult due to having small pores and high surface area (Balba et al., 1998). Clays can also catalyze humic acid formation and protect organic materials from decomposition within aggregates (Stott and Martin, 1990). Bioremediation of clays is therefore a challenging task. The ratio of external to total surface area of soil particles is also an important factor since only external surfaces are accessible to microorganism (Kwok and Loh, 2003).

The effect of soil type is among the less investigated factors affecting bioremediation. The aim of the present study is to determine the role of soil type and moisture content in the bioremediation of highly contaminated soils. The effects of soil type and moisture content have been investigated using a two factor factorial experimental design.

2. Materials and methods

2.1. Soil

Four different types of soil were selected. Sandy soil containing 100% sand (type I), clay soil containing more than 95% clay (type II), coarse grained soil containing 68% gravel and 32% sand (type III), and coarse grained soil with high clay content containing 40% gravel, 20% sand, and 40% clay (type IV). The soils were categorized based on the Unified Soil Classification System (USCS). Soils types I,II, and III were obtained from the suburb of Yazd, Iran (31.8972° N, 54.3678° E), and soil type IV was obtained from the suburb of Kerman, Iran (30.2907° N, 57.0679° E). The soils were stored in closed buckets at room conditions before the tests. An investigation on the origin of the soils indicated negligible organic content. The initially clean soils were contaminated with gasoil 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, and the soils were stored for the next step. The gasoil was obtained from Bandar Abbas refinery (Table 1).

2.2. Design of experiment for bioremediation

A full factorial experimental design was applied to investigate the effects of soil type, and moisture content on bioremediation. Soil type in four levels as mentioned above, and moisture content in two levels of 10 and 20% were investigated.

The experiment contained 8 runs which were performed in duplicate. 30 kg soil was used for each run. The soils were placed in buckets.110 g $(NH_4)_2SO_4$ and 108 g KH_2PO_4 were added to each bucket as nutrient supplements. The chemicals were purchased from Kiankaveh Azma pharmaceutical & chemicals complex Inc. These amounts resulted in the approximate ratio of 100:1:1 for Carbon: Nitrogen: phosphorous in soils at the beginning of the bioremediation process. The amount of $(NH_4)_2SO_4$ was considered lower than usual to avoid possible excessive osmotic pressure in the microenvironments of the soils.

A group of unidentified petroleum degrading microorganisms were used in this research. The microorganisms were isolated from a petroleum contaminated soil, previously undergone a bioremediation process. The microorganisms were grown in a basal mineral medium with the composition presented in Table 2, and with gasoil as the substrate. 500 mL of this microbe containing medium was used as the inoculums for each bucket. The moisture content of the soils was measured on monthly basis and adjusted with tap water. The soils were blended thoroughly after the addition of water.

Due the low removal percentage of TPH in clay soil, in a complementary experiment, the soil was subjected to more frequent water adjustment and mixing. In this experiment, two factors were examined: water content and mixing. Water content was examined at five levels of 5, 10, 20, 30, and 40%. Thorough mixing of the soil every 48 h after moisture adjustment was another factor (versus intact samples during bioremediation). The experiment was designed as a two factor factorial experiment having 10 runs. The runs were performed in duplicate. The samples weighed 500 g. For this experiment the bioremediation continued for one month.

2.3. Quantification of residual TPH in soil

For quantification of residual TPH is soil, samples (2 g) were taken and dried in an oven at the temperature of 70 °C for 5 h. The TPH content of the soil samples were extracted by Soxhlet apparatus and quantified based on the EPA Method 9071B (EPA, Method 9071B, 1998). A blank test was done to determine the efficiency TPH

Table 2
Composition of the basal mineral medium used for the initial microbial growth.

Compound (g L^{-1})	$CaCl_2 \cdot 7H_2O$ (0.04)	$MgCl_2 \cdot 7H_2O$ (0.2)	K_2HPO_4 (4.3)	KH_2PO_4 (3.4)	$(NH_4)_2SO_4$ (4)	$FeSO_4$ (0.03)
Compound (g L^{-1})	$MnCl_2$ (0.001)	$NaMoO_4$ (0.00175)	$CuSO_4$ (0.00015)	H_3BO_3 (0.000375)	$ZnSO_4$ (0.0017)	

Table 3
Removal percentage of TPH in soil after 270 days of bioremediation.

Moisture	Soil Type			
	Sandy soil	Clay soil	Coarse grained soil	Coarse grained soil with high clay content
10%	63%	23.5%	62.5%	65%
20%	70%	17%	57%	66.5%