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Hydrocarbon Bioremediation Efficiency by Two Indigenous Bacterial Strains in Contaminated Soils

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Abstract: Some industrial activities may lead to hydrocarbon contaminations of soil. Bioremediation of these contaminants is more effective than the other physical-chemical remediation methods. Therefore, the objective of this study was to investigate the efficiency of two indigenous bacteria in bioremediation at optimal condition. These bacteria were isolated from oil-contaminated soils. The experimental section was carried out thorough three steps: (i) Isolation and purification of indigenous bacterial from contaminated soils of south Tehran refinery. (ii) Preparation of the soil, which was used as a medium. (iii) Determination of biological removal of gasoil from soil. Results indicate that in optimal environmental conditions (temperature, 27±2°C, humidity of 60% WHC and daily aeration), bacterial isolates were able to degrade, about 78% of gasoil during the period of 45 days. This rate of removal seems to be acceptable, regarding to the short period of the experiments.

Key words: Bioremediation · Petroleum Contaminated-soils · Hydrocarbon Compounds · Oil-degrading Bacteria

INTRODUCTION

Hydrocarbon contamination is one of the most common types of contaminations in soil and aqueous ecosystems [1]. Industrial activities, is the most important source which contaminates the environment with these toxic and dangerous hydrocarbon compounds [2]. Despite all controls, petroleum hydrocarbons enter to the environment, during either excavation or transfer operations [3]. The most important environmental contaminants, which are common, include petroleum, gasoil, solvents (chlorinated solvents and BTEX[1]), PAHs[2] and PCBs[3] [4]. These organic compounds are recalcitrant and toxic. Therefore, will be harmful if enter to water resources [5]. Accumulation of these compounds in the environment is a serious threat for human health, organisms and bio-ecosystems [6]. Nowadays, bioremediation or microbial remediation is the most common technology in reclamation of the soils, which are contaminated with petroleum hydrocarbons. Bioremediation technology has been applied in the most contaminated sites all over the world [7]. Since bioremediation achieved noticeable successful results overcoming "Exxon Valdez oil spill" it considered as an effective and low cost method for environmental organizations [8]. Bioremediation is a method that employs potential of microorganisms to increase the rate and amount of degradation of contaminants; hence, it is a valuable tool to remove environmental contaminations [9]. A large number of bacteria, which have the potential of degrading petroleum hydrocarbon as a sole source of carbon, are recognized and isolated [10]. In addition, results of many studies show that bacteria have more degradation potential than fungi. Development and

[1]Benzene, Toluene, Ethylbenzene and Xylene
[2]Polycyclic Aromatic Hydrocarbons
[3]Poly Clorinated Biphenils

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enhancement of this technology require controlling complicated microbial mechanisms in degrading contaminated compounds and accelerating the remediation process [10]. New methods to amend contaminated soils by certain bacteria have been established such as inoculation of soils with indigenous bacteria of the same regions, which they are isolated and purified. Thus, the objective of this study was to determine the ability of degradation and remediation of two indigenous bacteria isolated from petroleum contaminated sites. In addition, this work aimed to study their biological removal efficiency in operational optimum condition. Two bacteria were isolated and purified from contaminated soils in south of Tehran refinery. Gasoil was also used as a model compound for hydrocarbon contaminants because gasoil is one of the widely used petroleum products in Iran and many other countries. Besides, large amount of gasoil is generally stored in underground reservoirs and fuel station, therefore, leakage of these reservoirs can contaminate surrounding soil and underground water.

MATERIALS AND METHODS

Three steps have been designated which were as follows:

- Isolation of indigenous bacteria for remediation of hydrocarbon contaminated soils; evaluation of their efficiency in degradation of gasoil; and finally selection of superior strains.

At south of Tehran refinery, six soil samples were obtained from the sites, which were contaminated with oil, apparently. The samples were kept in labeled closed jars in a cool box and transferred to the laboratory immediately. Then during three stages of growth test, gasoil degrading superior bacteria were selected:

The first stage; isolation in solid selective culture of Soil Extract/Agar media [11]. The second stage; study of variations in OD[4] in liquid mineral media with gasoil as the source of carbon [11, 12]. The third stage; Determination of respiration rate for the superior strains in a media with gasoil as the source of carbon [13]. At the end of these stages, two bacterial strains were isolated and purified. For typing and grouping isolated bacteria, some tests such as Oxidize test, Catalyze test, Mobility and Grams staining test, were fulfilled. All these experiments were performed based on standard methods of microbiology [14].

- Preparation of soil.

Sandy clay loam soil was sampled from Qazvin highway, air-dried and sieved by 2 mm sieve. Then for determining some physical and chemical characteristics, a series of experiments and measurements were fulfilled. All of the methods were based on standard methods [15]. Based on standards of soil P:N:C ratio, for optimal growth of bacteria in bioremediation operations (1:5:100), deficiency of these elements were compensated in the soil. It was supplied by addition of K_2HPO_4 and NH_4NO_3 [16].

- Determining removal rate of gasoil from soil by two superior isolates.

At this stage, 600 gr soils was weighted and transferred to a plastic flask. Then the soil was contaminated by gasoil (to the amount of 4% w/w). Finally, the contaminated soils were inoculated by 11 ml of suspension of isolated bacteria with the population of 3×10^9 bacterial number/ml. Soil moisture were kept at 60% WHC[5] during the experiment. The experimental units were incubated at 27±2°C for 45 days. Two factors were daily controlled:

- Soil manual mixing, to provide the optimal aeration for bacterial growth.
- Addition of water by atomizer, to keep humidity in a constant level.

After 45 days, 10 gr of contaminated soil was sampled and the residual gasoil was measured in each of the experimental units. “Normal hexane” was used as an extractor solvent. For each of the samples, 50 ml of normal hexane was used and then it was shaken for two hours in 200 rpm. Afterwards samples were centrifuged for 10 minutes in 500 rpm. The amount of residual gasoil in samples was measured by the method of “EPA 413.1” [16]. This experiment was carried out with three treatments, one treatment for each of the isolates and another one with the mixture of two isolates. Each treatment had three replications. Experimental design was “Completely

[4]Optical Density
[5]Water Holding Capacity

Random Design" (CRD). Mean comparison of the treatments was carried out by Duncan's multiple range test (P<0.01).

RESULTS AND DISCUSSION

- After three stages mentioned, two isolates (BJ.1 and BM.1) introduced as the superior and more efficient bacteria in degradation of gasoil in contaminated soils of southern Tehran refinery. Characteristics of these two isolates were illustrated in Table 1.
- Some physical and chemical characteristics of the soil, which was used as a bed, were measured. The characteristics of the soil are given in Table 2 and the results of particle size distribution of the soil were illustrated in Fig. 1.
- After measuring residual amount of gasoil in each experimental unit and subtracting it from the initial amount of gasoil (4% weight), "Biological Elimination" of gasoil was calculated. Inoculation of BJ.1, BM.1 bacteria and the mixture of them after 45 days could decrease the amount of gasoil from

Table 1: The characteristics of superior strains

Strain	Macroscopic characteristics	Microscopic characteristics	Mobility test	Grams staining test	Catalase test	Oxidase test
BJ.1	Smooth edge mucoid and milky color	Cocco bacil	-	-	-	-
BM.1	Smooth edge mucoid and milky color	Small cocci	-	-	-	-

Table 2: The results of some physical and chemical characteristics of the used soil

EC (ds/m)	0.223
pH	8.2
O.M (%)	0.16
O.C (%)	0.097
F.C (%)	32.23
N (%)	0.0043
P (mg/kg)	13.24

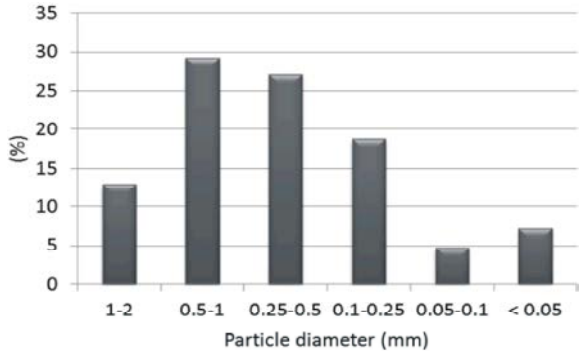

Fig. 1: Soil particle size distribution

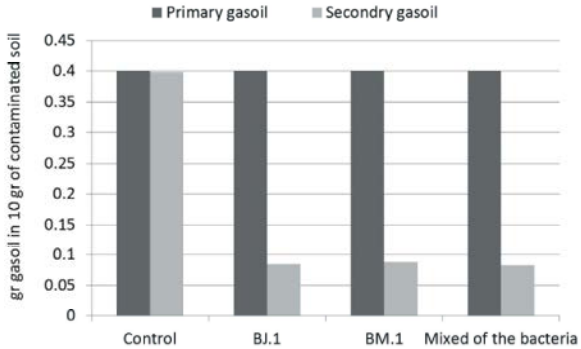

Fig. 2: Gasoil biological removal by bacterial strain

initial 0.4 gr to 0.084 gr, 0.087 gr and 0.082 gr respectively. Comparison of the mean by Duncan multiple range test illustrates that BJ.1 treatment, BM.1 treatment and the mixture (with that bacteria) treatment differs significantly in $P<0.01$ with control treatment. However, there is no significant difference between these three treatments in $P< 0.05$. It means that the efficiency of both two BJ.1 and BM.1 bacteria and the mixture of them to remove gasoil is approximately the same (Figure 2). Decrease in amount of gasoil is due to the bacterial consumption of gasoil as a carbon source for their growth. However, the amount of gasoil was decreased in control treatment after 45 days. It is probably due to spontaneously degradation of hydrocarbons in the soil. The results illustrate that regarding to the mentioned environmental condition (the temperature of 27±2°C, moisture of 60% WHC and daily aeration) BJ.1 and BM.1 bacterial species and the mixture of them could degrade and eliminate 78.36, 77.58 and 78.87 percent of gasoil after 45 days respectively. In addition, previous studies [12] show that amount of oil-contaminant in amended soil with bacteria, can be decrease to 15 % of its prior amount during 5 weeks. There are several studies with the same results as the results of current study about degradation and elimination of hydrocarbon contaminants by bacterial species [17, 18]. Results demonstrate that using existing laboratory facilities in the country and our indigenous microorganisms, it is possible to remediate soil, which contaminated with petroleum hydrocarbons. Bioremediation using indigenous microorganisms is one of the most effective and efficacious method, which has not principally any harmful environmental effects. In addition, Yang *et al.* [19] in 2009 have expressed that bioremediation is the best option for remediation since it is effective and economic in removing oil, with less environmental adverse effects.

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