



## RESEARCH PAPER

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## Bioremediation of gasoil 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. Biological options are described by the term bioremediation, 'the elimination, attenuation or transformation of polluting or contaminating substances by the use of biological processes, to minimize the risk to human health and the environment' Therefore, the objective of this study was to investigate the efficiency of two autochthonous bacteria in bioremediation at optimal condition. These bacteria were isolated from oil-contaminated soils, southern Tehran refinery. 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 for bioremediation. (iii) Determination of biological removal of gasoil from soil. Results indicated that in optimal environmental conditions (temperature,  $27 \pm 2$  °C, humidity of 60% WHC and daily aeration), bacterial isolates were able to degrade, about 93% of gasoil during the period of 45 days. This rate of removal seems to be acceptable, regarding to the short period of the experiments.

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## Introduction

Petroleum refining produces large amounts of effluents that are toxic and result in environmental pollution of receiving bodies of water and soils (Idise *et al.*, 2010). Such contaminated habitats lose their capability to support both plant and animal life and thus constitute public health and socioeconomic hazards as well as pose serious aquatic toxicity problems (Okerentugba and Ezeronye, 2004). One of the best approaches to restoring contaminated soil is to make use of microorganisms able to degrade those toxic compounds in a bioremediation process (Bento *et al.*, 2005). Many laboratory and field tests have demonstrated that the biological methods for soil remediation could be a cost-effective and environmental friendly technology to treat organic contaminants, particularly for petroleum hydrocarbon contaminated soils (Banks *et al.*, 2003, Mathew *et al.*, 2006). In contrast to physical and chemical methods, biological options generally maintain soil integrity with respect to its chemical and biological elements (Andrews *et al.*, 2013). In-situ bioremediation is the application of biological treatment to clean up the hazardous contaminant (Damborský *et al.*, 2000). In-situ bioremediation could be carried out either by the autochthonous or allochthonous organisms or both, through seeding (Ajayi *et al.*, 2008). 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 (Idise *et al.*, 2010). Adapted microbial communities usually have high proportions of hydrocarbon degraders that can respond to the presence of hydrocarbon pollutants. Improvement in the ability of microorganisms to degrade a pollutant could be achieved through modification of the environment or the organism (Idise *et al.*, 2010). Estimation of the presence and activities of organisms with abilities to metabolically utilize a contaminant is among the key parameters of the process design and its optimization as well as process (Damborský *et al.*, 2000). Thus, the objective of this study was to investigate the degradation ability 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.

## Materials and methods

Three steps have been designated which were as follows:

1. 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 (Ilyina *et al.*, 2003). Concentration of degrading organisms will estimated from the number of colonies will grow on agar plates supplied with contaminant as the sole carbon source (Damborský *et al.*, 2000)

The second stage; study of variations in OD in liquid mineral media with 4% gasoil as the source of carbon (Ilyina *et al.*, 2003, Márquez-Rocha *et al.*, 2001, Idise *et al.*, 2010)

The third stage; Determination of respiration rate for the superior strains in a media with gasoil as the source of carbon (Alef and Nannipieri, 1995). 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 (Table 1). All these experiments were performed based on standard methods of microbiology (Gerhardt *et al.*, 1981).

**2.** Preparation of uncontaminated soil for bioremediation media.

The selected soils for bioremediation media in this study, were uncontaminated Sandy clay loam subsoil's from typical areas around Tehran. Soils were air-dried, and sieved by 2 mm sieve. Then some Physicochemical properties (Table 2) were determined as described: soil pH, soil moisture (field capacity percent), soil salinity (electrical conductivity), soil organic matter & soil organic carbon, total nitrogen, plant available phosphorous and also soil particle size distribution. All of the methods were based on standard (Page, 1982). 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  $NH_4NO_3$  (Zhu *et al.*, 2001, Naji rad *et al.*, 2010).

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

At this stage, 540 gr soils and 60 gr sawdust were weighted and transferred to a plastic container (35cm × 20cm and 13cm deep). Then the soil was contaminated and mixed with 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  $3 \times 10^9$  bacterial number/ml. Soil moisture were kept at 60% WHC during the experiment. The experimental units were incubated at

$27 \pm 2$  °C for 45 days and two factors were daily controlled: \* Soil manual mixing, to provide the optimal aeration for bacterial growth and \*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" (USEPA, 200, Eaton and Franson, 2005). 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 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 & BM.1) introduced as the superior and more efficient bacteria in gasoil degradation in contaminated soils of southern Tehran refinery. Characteristics of these two isolates were illustrated in Table 1.

**Table 1.** The characteristics of superior strains.

Strain	Macroscopic characteristics	Microscopic characteristics	Mobility test	Grams staining test	Catalase test	Oxidase test
<i>BJ.1</i>	smooth edge mucoid, & milky color	Cocco bacil	Negative	Negative	Negative	Negative
<i>BM.1</i>	smooth edge mucoid, & milky color	Small cocci	Negative	Negative	Negative	Negative

Some physical and chemical characteristics of the soil, which was used as a bed (media), 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 and Table 3.

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

Characteristics	Value
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

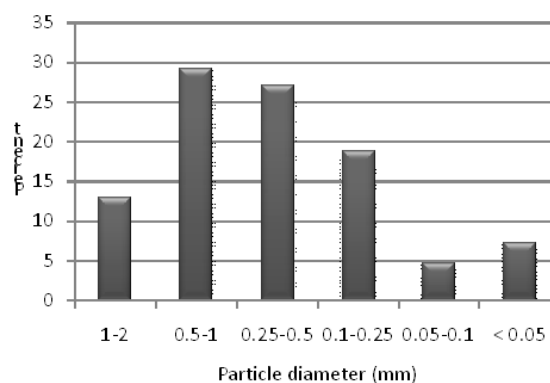
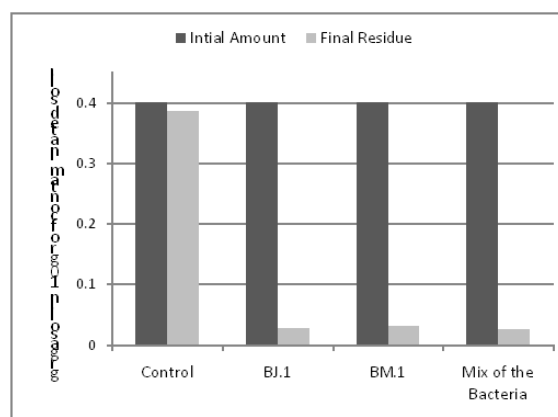
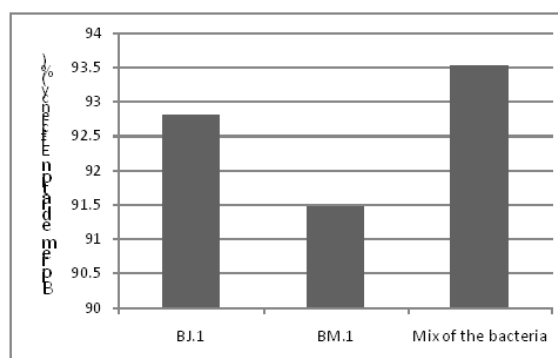
**Table 3.** The results of Soil particle size distribution.

particle size (mm)	percent
< 0.05	7.25
0.1 - 0.05	4.7
0.25 - 0.1	18.75
0.5 - 0.25	27.15
1 - 0.5	29.2
2 - 1	12.95

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 initial 0.4 gr to 0.028 gr, 0.031 gr and 0.025 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 in removal of gasoil is approximately the same (Fig.2).

### Discussion

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  $27 \pm 2$  °C, moisture of 60% WHC, and daily aeration) BJ.1 and BM.1 bacterial species and the mixture of them could degrade and eliminate 92.80, 91.47 and 93.53 percent of gasoil after 45 days respectively (Fig.3). In addition, previous studies show that amount of oil-contaminant in amended soil with bacteria, can be decrease to 15% of its prior amount during 5 weeks (Márquez-Rocha *et al.*, 2001). There are several researches with the same results as the results of current study about bioremediation of hydrocarbon contaminants by bacterial species (Ajayi *et al.*, 2008, Yang *et al.*, 2009, Idise *et al.*, 2010)

**Fig. 1.** Soil particle size distribution.**Fig. 2.** gasoil biological removal by bacterial strains.**Fig. 3.** Bioremediation Efficiency of bacterial strains.

Monitoring the indigenous microorganisms within a contaminated site is the simplest approach for cleanup of hydrocarbons (Deviny and Chang, 2000, Najirad *et al.*, 2010). This efficiency could be explained by the autochthonous adaptation with their contaminant hydrocarbon habitat that allows microorganisms to be physiologically compatible to digest and degrade the contaminant (Bento *et al.*, 2005). Results demonstrated that using existing laboratory facilities in the country and our autochthonous 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.

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