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Bioremediation of gasoil by indigenous bacterial strains

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ABSTRACT: Petroleum refining industries produce large amounts of toxic effluents, causing environmental pollution. Iran is an oil-rich country that encounters oil pollution in its soil and water. Bioremediation of these pollutants is an appropriate solution to tackle them, compared to physical and chemical remediation methods. There are some factors that increase the rate of biodegradation; therefore, this study aims to determine the rate of gasoil bioremediation by two indigenous bacterial isolates (from oil-contaminated soils of an oil refinery south of Tehran) in two different media, namely soil and soil-sawdust mixture. The two superior indigenous bacteria has been isolated through three steps with results indicating that in an optimal environmental condition (temperature= 27±2 °C, humidity of 60%, water holding capacity, and daily manual aeration), bacterial isolates are able to degrade about 78.87% and 93.53% of gasoil during 45 days in soil and soil-sawdust mixture media, respectively. These results imply the role of sawdust in improving aeration, water holding capacity, and-consequently- increasing bioavailability of gasoil to bacteria.

Keywords: biodegradation, hydrocarbon compounds, oil contamination, sawdust.

INTRODUCTION

Iran is an oil-rich country that produce large amounts of petroleum and its derivatives annually, in which a significant amount of petroleum enters the environment. For instance, studies have shown that there is petroleum and heavy metal contamination in sediments of Persian Gulf (Vaezi et al., 2015). Among the sources of petroleum production, petroleum-refining industries generate the largest quantities of oil sludge, which consists of hydrophobic and other substances (Couto et al., 2010). Concentration of these chemicals in the environment causes a serious threat to human health, organisms, and bio-ecosystems (Mirsal, 2008).

Therefore, one of the most important challenges, facing the Environmental Protection Agency, is to remediate contaminated sites. Physico-chemical treatments can be employed for soil cleanup, though they are extremely expensive (Ouyang et al., 2005), and they damage soil structure and/or utilize organic solvents, harmful for the environment. In contrast, biological remediation treatments

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are cost-effective approaches that rehabilitate soil structure (Couto et al., 2010). One of the best approaches for remediation of contaminated soil is to use microorganisms, which are able to degrade these toxic compounds (Bento et al., 2005).

Bioremediation is a method that uses microorganism's potential to increase the rate and amount of contaminant degradation. Ideally, the contaminant is the sole source of energy, ensuring that only those microorganisms, consuming it, will grow (Devinny & Chang 2000). Degradation of hydrocarbon contaminants can be enhanced by the inoculation of contaminated soils with microbial consortia or single isolates, known to be capable of degrading hydrocarbons (Richard & Vogel 1999; Bento et al., 2005). Many laboratory and field tests have demonstrated that the biological methods for soil remediation could be a cost-effective and environmentally-friendly technology to treat organic contaminants, particularly petroleum hydrocarbon, contaminating the soil (Mathew et al., 2006). There are also some studies in Iran that have shown the feasibility of bioremediation and biodegradation for removal of hydrocarbons from the contaminated soils (Najirad et al., 2012; Farahani et al., 2010; Amini et al., 2017; Niazy et al., 2016).

Biodegradation could be carried out either by autochthonous or allouchthonous organisms, or by a combination of both through seeding (Najirad et al., 2013). New methods have been developed to amend contaminated soils by certain bacteria, such as soil inoculation with indigenous bacteria of the same regions, isolated and purified during subsequent steps (Niazy et al., 2016). Adapted microbial communities usually have high proportions of hydrocarbon degraders that can respond to the presence of hydrocarbon pollutants. Improving the microorganisms' ability to degrade a pollutant could be achieved through modification of bacterial growth conditions.

Therefore, the objective of this study is to investigate the ability of two indigenous bacteria, isolated from oil-rich areas in gasoil degradation. The effect of two types of media, namely soil and soil-sawdust, has also been investigated.

MATERIAL AND METHODS

At first, six soil samples were selected from the sites which were likely to be contaminated. Hydrocarbon contamination was clearly visible on the soil surface in these sites. The samples were kept in labeled closed jars in a refrigerator and transferred to the laboratory. Soils were air-dried, and sieved through 2 mm sieve. Then some Physico-chemical properties (Table 1) were determined as described: soil pH, soil moisture (field capacity percent), soil salinity (electrical conductivity), soil organic matter and soil organic carbon, total nitrogen, plant's available phosphorous, and soil particle size distribution. All of the methods were in accordance with the standard (Page, 1983). Based on determined standards of soil, the ratio of C:N:P to optimum bacteria growth for bioremediation operations (1:5:100), deficiency of these elements were evaluated in the soil and was supplied by adding Di Potassium Hydrogen Phosphate (K_2HPO_4) and Ammonium Nitrate (NH_4NO_3) (Zhu et al., 2001). The nutrients were added and dissolved in water to facilitate the entrance/dissolution on N and P in the soil matrix (Couto et al., 2010).

Table 1. The characteristics of superior strains isolated form the contaminated soil

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

Then, gasoil degrading superior bacteria were isolated and selected through three steps of growth tests. The isolation was carried out in solid selective culture media of Contaminated-Soil Extract/Agar with hydrocarbon as the sole source of carbon (Bardi et al., 2000; Ilyina et al., 2003). Then, variations of bacterial optical density were measured, using spectrophotometer in liquid mineral media with gasoil as the source of carbon (Pontecorvo 1949; Bardi et al., 2000; Ilyina et al., 2003; Márquez-Rocha et al., 2001; Idise et al., 2010; Karamalidis et al., 2010). Then, bacterial respiration was measured for superior strains in a media with gasoil carbon source (Alef & Nannipieri 1995; Sabaté et al., 2004). At the end of these three steps, two bacterial species were isolated and purified. For typing and grouping isolated bacteria, macroscopic and microscopic experiments were conducted, such as Oxidize test, Catalase test, Mobility, and Grams staining test, all in accordance to microbiological standard methods (Gerhardt & Microbiology, 1981).

Finally, the contaminated media were inoculated with 11 ml suspension of bacteria with a population of 3×10^9 bacterial number/ml and the media moisture was kept at 60% WHC during the experiment, as it was experimentally observed that higher amounts of the mentioned moisture cause a muddy medium and it would prevent appropriate aeration.

The experimental units were incubated in a temperature equal to 27±2 °C for 45 days with the following two factors, controlled daily:

1. Soil manual aeration was done, using a garden hoe to provide the factor of optimum aeration for bacterial growth (Couto et al., 2010).

2. Bacterial essential water was added by an atomizer to keep humidity.

The containers were treated differently, as described in Table 2. In this step, 12 plastic containers of the same size were chosen. They were filled up as the following: to the six of the containers 600 g soil and to the rest 540 g soil and 60 g sawdust were added. The media were contaminated with gasoil 4% (w/w). As far as gasoil density was 0.89, the amount of 4% (W/W) gasoil for 600 g of the experimental media was equal to 24 g or 26.7 ml gasoil. Then 600 g experimental media got well-homogenized with 26.7 ml gasoil to final concentration of about 4 g contaminant per 100 g media.

After 45 days, an amount of 10 g of contaminated media was weighted and the residual gasoil amount was measured in the experimental units. In this research, "Normal Hexane" was used as gasoil extraction solvent and for each 10 g of the media, 50 ml Normal Hexane was used and was shaked for 2 hours in 200 rpm, then to be transferred to centrifuge tube and centrifuged for 10 minutes in 500 rpm. Then the amount of residual gasoil in samples was measured via "EPA 413.1" Method (Zhu et al., 2001; Eaton et al., 1998).

This experiment was conducted by both of the bacterial strains and a control treatment (three treatments) in two different media (soil and soil-sawdust mixture). It was carried out in triplicate as a Completely Random Design (CRD). Mean comparison of the treatments was done by Duncan's multiple range test ($P\leq0.01$).

Table 2. Experimental design, showing the composition of the media used as the bed for bioremediation test

Experimental units*	Inoculation of both bacterial strains
SM	+
SMC	-
SSM	+
SSMC	-

* SM: Soil Media, SMC: Soil Media Control, SSM: Soil/Sawdust Media, SSMC: Soil/Sawdust Media Control

RESULTS

In first step, to study the ability of oil-degrading bacteria to degrade gasoil, a sum of 41 isolates was purified in solidified-selective media of Soil Extract/Agar upon their growth rate and maximum colony diameter for 20 days.

Then, in the second step, four species were selected based on the turbidity of their culture as an indicator of growth in liquid mineral media (with a proportion of 7% volume gasoil concentration as a source of carbon), during 15 days.

In the third step, based on the mineralization method (CO_2 production and measurement of the respiration, related to each species of bacteria) two species were selected during six weeks as the superior and more efficient ones to degrade gasoil in contaminated soils of southern Tehran refinery. Table 1 presents the characteristics of these two isolates, while Table 3 gives some physical and chemical characteristics of soil, with Figure 1 illustrating the results of particle size distribution. The soil texture was "sandy clay loam" and the particles, the diameter of which was below 0.1 mm, comprised minor portion of soil particles. If otherwise the soil could not meet the optimum requirements for bacterial growth.

Table 3. The results of some physical and chemical characteristics of the soil, used as the bed for bioremediation test

Characteristics	Value
Electrical Conductivity (dS/m)	0.223
Organic Matter (%)	0.16
Organic Carbon (%)	0.097
Field Capacity Moisture (%)	32.23
Nitrogen (%)	0.0043
Phosphorous (mg/kg)	13.24
pH	8.2

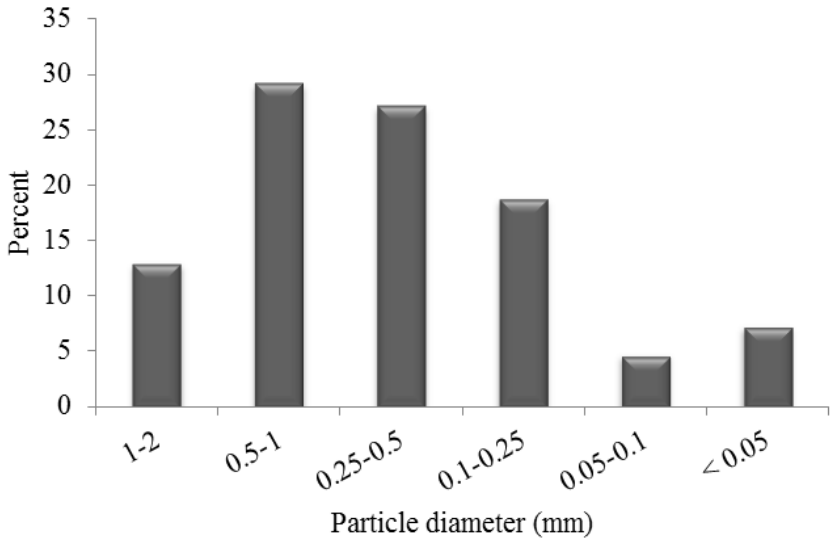

Fig. 1. Particle size distribution (ranging from less than 0.05mm to 2mm) of the studied soil

Once the residual gasoil was measured in each experimental unit, and was subtracted from the first amount of gasoil contamination (4% [w/w]), decreasing in amount, the "Biological Elimination" of gasoil was obtained. Throughout the method, 10 g of contaminated soil was weighted and the residual gasoil measured, showing that the preliminary contamination for 10 g of medium was equal to 0.4 gr. So, we expect the amount of residual gasoil in each experimental unit, inoculated with bacteria, is less than 0.4 g and in control treatments (without bacteria) it would be 0.4 or approximately 0.4. The residual amount of gasoil in the treatments differed significantly.

Mean Comparison by Duncan's multiple range test illustrates that there is a significant difference between the inoculated treatments and the control ($P \leq 0.01$). The significant differences were also seen between the soil and the soil-sawdust media ($P \leq 0.01$). Figure 2 shows the residual of gasoil in the experimental media after 45 days. Inoculation of both bacterial strains could decrease the amount of contamination from 0.4 g, as preliminary gasoil to 0.0823 and 0.0253 g in soil and soil-sawdust mixture media, respectively. The amount of residual gasoil in control treatment (without bacteria) was approximately the same as the preliminary gasoil. According to Table 4, the amount of gasoil also decreased in control treatment after 45 days, probably due to spontaneous degradation. This amount was subtracted from the other treatments for final determination of gasoil biological elimination efficiency.

Table 4. Residual gasoil amount (g gasoil in 10 g of contaminated soil, after 45 days)

Mean residual gasoil amount	Treatment
0.0823^{b}	Inoculation on soil media
0.0253^{c}	Inoculation on soil-sawdust media
0.389^{a}	Control-soil media
0.386^{a}	Control-soil-sawdust media

The values with different alphabets are significantly different in $P \leq 0.01$

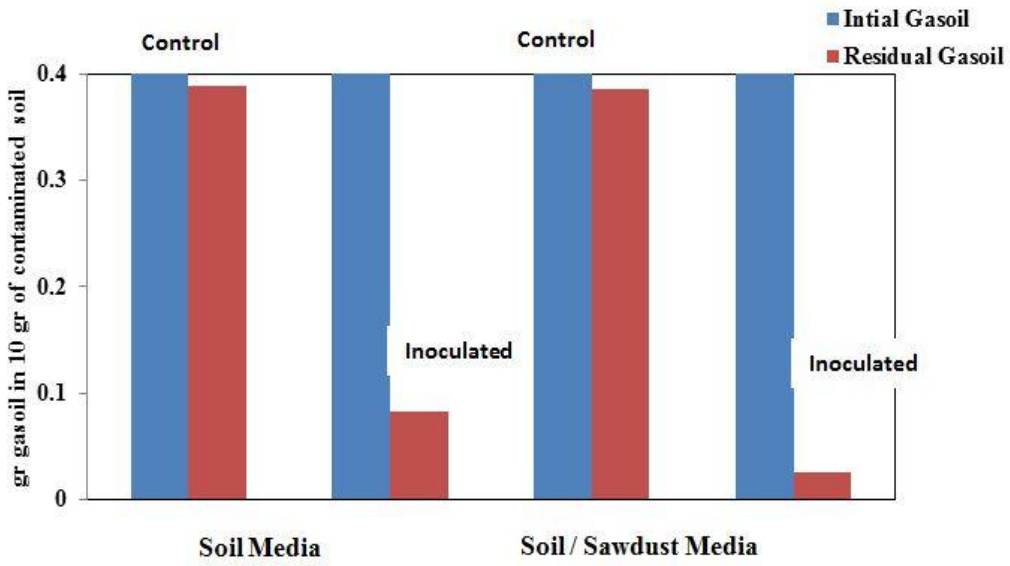

Fig. 2. Gasoil removal by bacterial species (Inoculated) compared to the control, in soil and soil-sawdust media, after 45 days

Also, previous studies (Márquez-Rocha et al., 2001) showed that the amount of oil contaminants with bacterial inoculation could plummet to 15% of its prior amount within five weeks (Najirad et al., 2012). Results in researches by Salanitro (2001), Xu and Obbard (2003), Ferguson et al. (2003), and Si-Zhong et al., (2009) are similar to those of the current study, concerning degradation and elimination of hydrocarbon contaminants by bacterial species. This efficiency could be explained by the autochthonous adaptation with their contaminant hydrocarbon habitat, allowing microorganisms to be physiologically compatible for digestion and degradation of the contaminant (Bento et al., 2005).

Figure 3 illustrates the effectiveness of gasoil bioremediation in different treatments. The efficiency of gasoil degradation by both bacterial strains was about 78.87% and 93.53% in soil and soil-sawdust mixture media, respectively. These results imply that sawdust might be involved in the degradation by: (1) aeration improvement, (2) increasing Water Holding Capacity (WHC), and (3) increasing bioavailability of gasoil to bacteria. The same results are shown in recent works (Thapa et al., 2012). Improving microorganisms' ability to degrade a pollutant could be achieved through modifying the environmental conditions for bacterial growth (Idise et al., 2010).

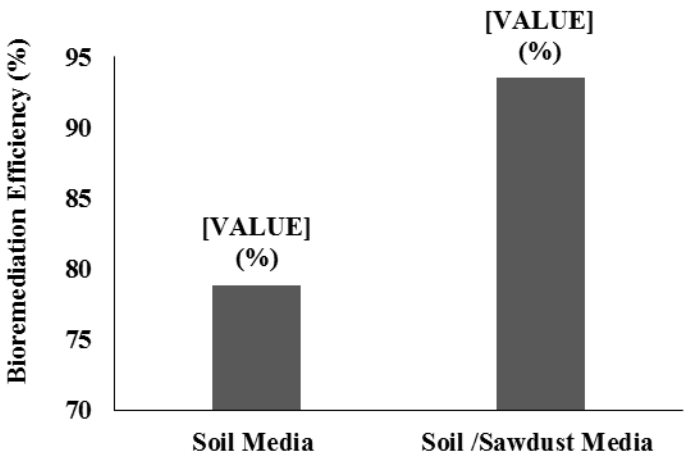

Fig. 3. The efficiency of gasoil bioremediation by both of the bacterial isolates in two different media (soil and soil/sawdust media)

CONCLUSIONS

The gasoil elimination in inoculated treatments is due to bacterial consumption of gasoil as a carbon source for their growth. Results illustrate that at the mentioned environmental condition (the temperature of 27±2 °C, Moisture of 60% WHC, and daily manual aeration) bacterial strains could degrade approximately 78.87% and 93.53% of gasoil in soil and soil-sawdust mixture media, respectively. Given the experiment duration (45 days), this seems to be an acceptable result. Gasoil contamination is decreased during 45 days to less than half of preliminary contamination amount in the media, inoculated with oil-degrading bacteria. Also soil-sawdust media is more appropriate than the soil media in gasoil bioremediation. It is probably because of the impact of sawdust in the improvement of environmental conditions for bacterial growth. Bioremediation with indigenous microorganisms is one of the most effective methods that has no harmful environmental effects. It is also the most effective method to degrade hydrocarbon contaminants in a short time.

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