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Biodegradation Assessment of Petroleum-Contaminated Soils by Natural Attenuation, Biostimulation and Bioaugmentation Methods on an Industrial Scale

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Abstract

This research study compares the biodegradation of petroleum hydrocarbons by natural attenuation, biostimulation and bioaugmentation methods by applying the Pseudomonas aeruginosa and Bacillus cereus bacteria in hydrocarbon-polluted land area of 1000 square meters consists of three distinct parts to compare the methods discussed above for 100 days. The petroleum-contaminated percentage of this area (w / w) is 0.5%. The extraction and determination of petroleum hydrocarbons percentage in contaminated soil was done by gravimetric method by liquid-liquid extraction method by hexane, dichloromethane and chloroform solvents. The results showed that the bioaugmentation technique has a higher ability to analyze the petroleum hydrocarbons contaminant that reduced about 82.33% of this pollution in the period of 100 days and biostimulation and natural attenuation methods decreased the hydrocarbon materials percentage to the rate of 40.32 and 12.11% respectively.

Keywords: natural attenuation, biostimulation, bioaugmentation, biodegradation, petroleum hydrocarbons

Introduction

Petroleum hydrocarbons are produced from the first phase of drilling to find oil until providing products to consumers. Petroleum hydrocarbons contaminations are mainly produced from two separate sources: first, the extraction and storage process of crude oil, second, the refining process. Petroleum contaminations generated from the first source can be stored at the bottom of oil storage tanks or on drilling equipment. Refining process produces more waste that is a complex mixture of liquid and solid phases. Biodegradation method is successfully used for purifying the environment that is contaminated to the carbon materials. However, little effort has been done to improve the petroleum hydrocarbons status. For that very little information exists about the growth power of the micro-organisms in these environments. When crude oil hydrocarbons enter into the environment, the lighter compounds evaporate and heavier compounds remain in the environment. The fate of hydrocarbons remaining in the environment is determined by moisture, wind, pH, and features such as concentration, their distribution method, structure, solubility, environmental condition and microorganisms in the nature. Solubility of hydrocarbons is the most important parameter in determining their destiny. Usually the solubility of organic compounds is different; some like polar materials (methanol) are soluble to any rate and others such as poly aromatic hydrocarbons have low solubility and usually attracted to the solid particles strongly. A part that is solution transferred by attraction and penetration and is more available to analysts. Metabolism of hydrocarbons in the nature is mainly driven by bacteria and fungi. Total percentages of heterotrophic hydrocarbons that normally decomposed are highly variable. Different families of bacteria and fungi have shown their ability in petroleum hydrocarbons analysis. In fact, 22 kinds of bacteria and 31kinds of fungi with the ability to analyze the hydrocarbons are collected from soils contaminated with petroleum hydrocarbons (Leahy & Colwell, 1990). Similarly, 25 types of bacteria

and 27 types of fungi were collected from the aqueous area of sea. It is reported that the following bacteria as a part of the community of bacteria grow in the petroleum hydrocarbons (Van hamme, Odumeru, &Ward, 2000), and most of the report is devoted to the Alcaligenes spp., Stenotrophomonas maltophilia, Yersinia spp., Bacillus spp., Micrococcus, while Pseudomonas spp. by producing Bio surfactant increases the oil solubility in water and thus becomes more accessible to the microorganisms (Rocha, & Infant, 1997). It is specified that other bacteria like Acinetobacter, Achromobacter, Aethrobacter Flavobacterium, Nocardia grow on petroleum hydrocarbons derived from crude oil or contaminated soils. Microorganisms were targeted from 1940for the purification and control of accidental falling of oil. After that, they were used for the soil contamination removal. As a result, more data was available on the ecological environment interaction between microorganisms and soil, water, and oil. Recently, advocates of preservation of natural resources give high priority to the problem of hydrocarbons contamination which is analyzed by organisms because it eliminates hydrocarbons and converts to carbon dioxide, water and biomass. They also cost less than other methods. Time is the biggest problem of this method and the process is slower than other methods (Baker, and Herson, 1994, Riser-Roberts, 1998). Ideal situation and additives should be optimized in order to stimulate the microbes to grow better. Different bacterial composition is used in purification of petroleum sludge of refinery that bacteria were selectively separated from contaminated hydrocarbon of soil or petroleum sludge. Van also conducted studies on the dynamics of microbial growth in petroleum sludge and results showed that bacteria possess different abilities to grow in the sludge (Van hamme, Odumeru, and Ward, 2000). Their growth was improved by adding nonionic surfactant. Lazer conducted studies on the ability of six bacteria to decompose the petroleum hydrocarbons. The experiments were performed on the laboratory scale and samples were grown under different conditions (Lazer, Voicu, Dobrota, Stefanesc, Sandulescu, and Petrisor, 1999). Mishra separated the bacteria from soil contaminated with petroleum hydrocarbons. These bacteria, which are called Alizaper were prepared as powder. 88% reduction in petroleum hydrocarbons were obtained by this method (Mishra, Lal, Jyot, Ranjan, Khanna, and Kuhad, 1999). Very little information is available about the use of microbes for purification of petroleum hydrocarbons in natural scale that is addressed in this study.

Materials and methods

Preparing the testing and sampling environment

This research was done in the soil environment of 300 cubic meters including three separate parts near Shiraz city that had been contaminated by the oil spill. In order to determine this mean pollution, initially it is well mixed by farming machine and more than 50 points were randomly taken as sample and to specify the mean amount of pollution it was sent to Lab. The studied area was divided into three parts which were separated by iron metal wall that was placed to a depth of 50 cm in the ground.

Liquid cultivation environment

In this experiment, in order to growth and reproduction of bacteria and preparing biostimuilation solution a special liquid cultivation environment (MSM) containing mineral salts is used and its compounds have been prepared from Merck company of Germany. These compounds are listed in Table 1.

Then the cultivation environment is autoclaved and is completed by 10m/lof 0.25% w/v solution, $MgSO_4.7H2O$, and 10 m/l of %1w/v solution, $(NH4)_2SO_4$, which were autoclaved as the main cultivation environment. Also, the public cultivation environment of BHI was used for amplification and maintenance of pure colonies of bacteria.

Table 1. The compounds of basic cultivation environment

compounds	Amount (g/L)	compounds	Amount (g/L)
K_2HPO_4	13/9	NaCL	1
KH_2PO_4	2/7	$NaNo_3$	1
Yeast extract	0/5		

Measuring the mean of total hydrocarbons materials (TPH) existing in the sample

The method explained by Mishra and colleagues was used to determine the total Petroleum Hydrocarbons existing in the samples. 1 gram of each sample was extracted in order and separated by 50 mL of hexane, dichloromethane and chloroform solvents. All extracted solutions were combined with each other and solvents were evaporated at room temperature under a nitrogen gas stream and the remaining material was weighed. Eventually, the secondary weight was mitigated of 1 gram and TPH was obtained.

$$\text{TPH Reduction } \% = \frac{\text{Initial weight-Secondary weight}}{\text{Initial weight}} \times 100$$

Microorganisms Selection

The bacteria of Pseudomonas aeroginosa (Figure1) and Bacillus cereus (2) isolated from soil contaminated with oily substances were used in this study which have high capability to remove these materials.

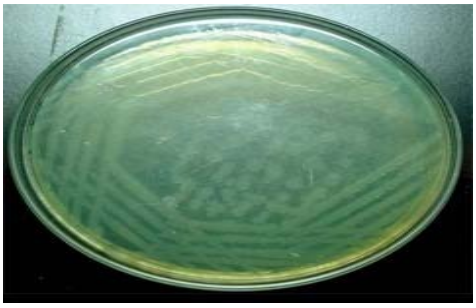

Figure 1. Pseudomonas aeruginosa

Figure 2. Bacillus cereus

Procedure

In this study, the techniques of natural attenuation, biostimulation and bioaugmentation were used to improve biological contamination of the oil. In the first technique (natural attenuation) contamination decomposition was completely evaluated naturally by inherent microorganisms and after 100 days. In Biostimulation method MSM nutrient solution is prepared and injected into the ground at a rate of 1 ml in 1 cubic meter in order to stimulate the inherent microorganisms and to

compensate the nutrient deficiencies for growth of bacteria. In bioaugmentation method intrinsic or non- intrinsic bacteria or both of them are used. Pseudomonas aeroginosa and Bacillus cereus bacteria were used as assistive bacteria to break down petroleum materials. First, these bacteria were reproduced in laboratory in completely sterile form and were injected into the ground along with the MSM solution. Due to the increased soil moisture and having homogeneous environment, the studied environment is irrigated per week 1 time by agricultural machine. After 50 and 100 days, the removal percentage of petroleum materials was determined by sampling from different parts of the earth and extracting TPH in the laboratory.

Results and discussion

Mean percentage of environmental oil pollution

In the first day of study the mean of samples taken by the method described by Mishra et al was specified by Liquid-Liquid Extraction Technique and the percentage of environmental oil pollution (w / w) was 0.5%.

Petroleum removal percentage

This study aimed to find a suitable method for the removal of petroleum materials with low operating costs and likes environment. Results of the experiment for 100 days showed that in the technique of natural attenuation the oil pollution removal rates is 12.11%, which was indicated that inherent bacteria due to poor condition or lack of nutrients are not able to analyze tenacity such as aromatics, asphaltene and heavy hydrocarbons with long-chain. In the biostimulation method, it was observed that due to the injection of nutrients into the soil and providing nutrients for the growth of bacteria and stimulating them to remove petroleum materials, pollution removal percentage was increased to 40.32% that this amount within 100 days is appropriate and acceptable from the environmental point of view. In the state of bioaugmentation, the removal percentage was increased to the high amount of 82.33% within 100 days. This removal amount is due to the existence of injected nutrients and non- intrinsic bacteria of Pseudomonas aeroginosa and Bacillus cereus with ability to remove most petroleum materials. Table 2 indicates the growth of these bacteria in MSM liquid environment and various hydrocarbon materials as carbon source. Also, the percentage of petroleum contamination removal in 50 and 100 days by three mentioned methods is shown is Table 2.

Table 2. The growth ability of bacteria in MSM solution along with various carbon source

Carbone Source	Pseudomonas aeroginosa	Bacillus cereus
n-hexadecane	***	**
Dodecane	**	**
Heptane	**	*
Naphtalene	**	**
Toluene	**	**
Asphaltene	**	*
Crude Oil	***	**

* Weak growth, ** Good growth, *** Very good growth

Table 3. The removal percentage of total petroleum contaminations (TPH) by Liquid-Liquid Extraction method

	The reduction percentage of TPH	
Techniques	After 50 days	After 100 days
Natural attenuation	8.21	12.11
Biostimulation	27.32	40.32
Bioaugmentation	59.62	82.33

Conclusion

Results indicated that Bioremediation technique was so suitable in the environmental problems solving and also had high ability in removing petroleum materials in the soil. The main features of this method includes liking environment and fixed costs and low operations

Also, it was observed that in bioaugmentation method the percentage of petroleum contaminations was reduced more compared to the other methods. It is necessary to note that this method can quickly prevent the leakage of contaminations into the groundwater and other areas and can analyze a great percentage of these contaminations in the short time. Petroleum materials are such as carcinogens, which is a serious threat to the health of humans and animals. Preventing the leakage of these substances into the environment is essential and requires appropriate management. In this study, according to the results of test and also due to the extensive contaminations created in Iran, it is suggested to utilize the biological improvement techniques to remove it.

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