Using Indigenous Microorganisms to Reduce Petroleum Contamination in Tehran Oil Refinery Contaminated Soil

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Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are widely distributed contaminants which have drawn increasing public health concern because of their mutagenic and carcinogenic properties. In addition to their natural occurrence, anthropogenic inputs from fuel combustion, pyrolytic processes, waste incinerators, domestic heaters and spillage of petroleum products have resulted in a significant accumulation of PAHs in the environment. Petroleum contaminated soil is currently treated using three types of processes: physical, chemical, and biological. Bioremediation is an attractive approach for cleaning up petroleum hydrocarbons, because it is simple to maintain, applicable over large areas, cost-effective and leads to the complete destruction of contaminants. Organic compounds are metabolized under aerobic or anaerobic conditions by biological processes of microorganisms. Bioremediation of contaminants can be accomplished by two methods, bioaugmentation and biostimulation. The process of bioaugmentation, as it applies to remediation of petroleum hydrocarbon contaminated soil, involves the introduction of microorganisms that have been cultured to degrade various chains of hydrocarbons into a contaminated system. The process of biostimulation introduces additional nutrients in the form of organic or inorganic fertilizers into a contaminated system, which increases the population of the indigenous microorganisms. In the field of biostimulation, nutrient supplementation for hydrocarbon degradation has traditionally focused on the addition of N and P. Because C is a major constituent of petroleum fuels, its traditional role in bioremediation research has typically been as an index to determine the amount of N and P that needs to be added to reach the optimal C:N:P ratio. The indigenous microorganisms of the contaminated soils, which are adapted to hard conditions, are the best microbial population for using in bioaugmentation. Various microorganisms have different requirements for using the carbon contents of pollutants as an energy source. Thus, the successful application of bioremediation technology to contaminated soil requires knowledge about the characteristics of the site and the parameters that affect the microbial biodegradation of pollutants. This study was carried out to isolate high diversity of bacterial strain with the ability of degrading multiple PAHs from Tehran oil refinery contaminated soil and obtain the optimized conditions of biodegradation of these pollutants in laboratory. The final objective was to compare the effectiveness of natural attenuation, biostimulation and bioaugmentation for PAHs degradation in soil.

Materials and Methods

Soil samples were collected in sterilized panels from four different geographical directions of a land next to the active units of Tehran oil Refinery in Iran. The soil properties of samples are provided in Table.1. The presence of PAHs contamination in the soil of this land was confirmed by solvent extraction (Dichloro methane) in an ultrasonic water bath and high HPLC analysis.

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Table 1:The physico-chemical characteristics of the soil samples

Soil sample	pH (1:1)	CECmeq/l	Organic Carbon%	Total N%	Total Pmg/l	Soil texture	Saturated soil moisture %
1	7.22	52.3	0.61	0.14	20.3	Clay	27.7
2	7.25	51.5	1.12	0.13	19.3	Clay	30.5
2	7.42	51.1	0.81	0.11	18.1	Clay	30.7
4	7.49	52.1	0.84	0.08	15.6	Clay	30.8
Mixture	7.41	52.10	0.73	0.13	19.1	Clay	29.9

Five grams of soil samples were inoculated into separate contial flasks containing 45 ml of Oil broth a substrate of petroleum, containing 5% of vacuum bottom in crude oil of Tehran Oil Refinery prepared and used as a source of PAHs. 500 µl of the substrate was served as the sole carbon and energy source for bacterial growth. The enrichment cultures were incubated aerobically at 37˚C on a rotary incubator at the speed of 180 rpm. Following visible growth, aliquots of the cultures were transferred to fresh Oil broth, also containing 500 µl of the substrate. To obtain PAHs degrading isolates, these enrichments were plated on Oil broth agar plates and sprayed with the same substrate used in the enrichment stage. Five isolated bacteria with approximately 10^7 cells were inoculated into separate contial flasks containing 10 ml of Oil broth with various concentrations of substrate: 1%, 5%, 10%, 20%, 30% and incubated aerobically at 30˚C on a rotary incubator at 180 rpm for 24h. The potential of PAHs biodegradation was estimated by measuring the optical density (OD) at 540 nm, after extracting the remaining substrate with 5 ml n-hexane. These experiments were also carried out changing other parameters: pH, nutrients, and temperatures, to obtain the optimal conditions of PAHs biodegradation. The parameters were ranging as follows: pH: 6, 6.5, 7, 7.5, 8; Temperature: 25, 30, 37; Nutrient: NH_4No_3, $(NH_4)_2So_4$, $(NH_2)_2Co$. Different ratios of C: N: P were 100:10:1, 100:5:1, 100:10:5, 100: 1:0.2. Finally, three treatments were carried out under control to compare natural attenuation, biostimulation and bioaugmentation for PAHs degradation in soil in laboratory. The contaminated soil sample was sprayed with the substrate, before being used for bioremediation studies, in order for the percentage of organic carbon to reach to 2%. The soils (500g) were placed in a set of glass pans with the volume of 1L. The soils were mixed weekly to provide sufficient air and oxygen. The pans were covered with Aluminum foil and kept at 30˚C in incubator. The soil was moisturized by the addition of 20 ml of sterile distilled water every week until the end of the experiment. The control was sterilized three times by autoclaving at 121˚C for 30 min. The soils were biostimulated by adding $(NH_4)_2So_4$ and K_2HPo_4 to give a concentration equivalent to a C: N: P molar ratio of 100:10:1 (the best result of optimizing experiments). The same conditions were provided in the bioaugmentation treatment, plus a microbial consortium from five selected species isolated to reach 10^8 microorganisms g-1 of soil. The blank sample was used to assess natural attenuation treatment. The contaminated soils for the treatments were sampled at 0, 1, 2, 3, and 4 weeks for determining of microbial population and concentration of PAHs. One-way ANOVA and LSD tests were carried out to determine whether, the percentage of PAHs biodegradation and microbial population differs significantly according to the type of the treatment.

Results and discussion

Five of the isolated samples with the highest rate of growth were selected for testing the effect of the concentration of the substrate on the PAHs biodegradation and further characterization. Morphological and biochemical characterization of PAHs revealed the following organisms: Pseudomonas sp., Micrococcus sp., and Bacillus sphaericus. The results showed that the best conditions for efficient removal of Poly Aromatic Hydrocarbons are pH of 7.5, temperature of 30˚C, and ratio of C: N: P equal to 100: 5: 1, 100 :10 :1, and 100: 5: 1 for NH_4No_3, $(NH_4)_2So_4$, $(NH_2)_2Co$ as sources of N, respectively. Percentage of biodegradation of PAHs was calculated according to following equation:

[(PAHs control – PAHs treatment)/PAHs control] × 100

Table 2 shows the Percentage of biodegradation of Total PAHs in the soil contaminated samples. Based on these results, the highest biodegradation rate (58.2–70.5%) of PAHs was observed in the contaminated soil when a consortium of the selected bacteria (Bioaugmentation) was added. The effect of nutrient addition (biostimulation) on the PAHs biodegradation rate was obtained to be 32.1– 45.7%, and the effect of natural attenuation was estimated to be 22.9–37.2%. Also, Fig. 6 shows the comparison of biodegradation rates of PAHs in different treatments. The bioremediation rates after 4 weeks of incubation in descending order are as follows: bioaugmentation (70.5) > biostimulation (45.7) > natural attenuation (37.2), which agree with the results obtained by other researchers. Data were subjected to variance analysis by using a single independent variable to determine that there are differences among the mean values. The results of statistical analysis showed the significant different ($p<0.05$) between bioaugmentation and the other types of treatments. This means that the additional consortium of the selected bacteria could increase the bioremediation rate of PAHs in soil. Further, the effect of inoculation of the microbial consortium and setting the C: N: P ratio is higher than the mere addition of the nutrients. No significant difference was observed ($p<0.05$) between biostimulation and natural attenuation, which means that the addition of nutrients had no significant effect on biodegradation rate of PAHs in soil.

Table 2: Percentage of biodegradation of Total PAHs in the soil contaminated samples

Weeks	Natural attenuation	Biostimulation	Bioaugmentation
1	22.9	32.1	58.2
2	30.1	38.8	64.7
3	33.5	43.3	68.9
4	37.2	45.7	70.5

Conclusions

Bioremediation of PAHs-contaminated soils can be accomplished by two methods, bioaugmentation and biostimulation. In bioremediation methods, careful adjustments of C: N: P ratio might be enough to optimize the conditions for microbial metabolism. The results of this investigation showed that, the best conditions for the efficient removal of PAHs are: pH of 7.5, temperature of 30°C, and the ratio of C: N: P equal to 100: 5: 1, 100: 10: 1 and 100: 5: 1 for NH_4No_3, $(NH_4)_2So_4$, $(NH_2)_2Co$ as sources of N, respectively. $(NH_4)_2So_4$ as a source of N, has the highest effect on the bacterial growth. The highest biodegradation rate of PAHs was observed in the contaminated soil when a consortium of the selected bacteria (Bioaugmentation) was added and the maximum bioremediation rate after 4 weeks of the incubation in descending order is: bioaugmentation (70.5%) > biostimulation (45.7%) > natural attenuation (37.2%). Based on the result of this study, it was concluded that increasing the incubation time would increase the amount of the degradation of PAHs and microbial population. However, the highest rate of degradation was observed in the first week.

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Key words

Poly Aromatic Hydrocarbons, Bioremediation, Contamination, Soil