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Bioremediation of total petroleum hydrocarbons in crude oil contaminated soils obtained from southeast Anatolia

Senem ÜSTÜN KURNAZ[1], Hanife BÜYÜKGÜNGÖR*[2]

[1]General Directorate of Turkish State Hydraulic Works, VII. Regional Directorate, 55209 Samsun, Turkey.

[2]Department of Environmental Engineering, Ondokuz Mayıs University, 55200 Samsun, Turkey.

*Corresponding author: hbuyukg@omu.edu.tr

Abstract: Oil spills are one of the most common types of soil pollution. Bioremediation is an attractive alternative method to physico-chemical remediation. It is well-known that bacteria and fungi are the principal petroleum degrading organisms. Hence, this study investigates the use of fungus *Aspergillus niger* to remove TPH (Total Petroleum Hydrocarbons) from soil polluted with crude oil obtained from Southeast Anatolia of Turkey, and accordingly the biodegradable of TPH in soil by *A. niger* biomass was investigated. The amount of TPH was measured before and after *A. niger* application. For this purpose, the biodegradation of TPH was applied to three different soil media, including (I) contain only *A. niger* in sterilized soil, (II) soil microorganisms - *A. niger*, and (III) only soil microorganisms. The experiment was conducted at 30°C for 30, 65 and 96 days without pH modification (original pH was 5.0). At the beginning and end of the experimental periods, the samples of soil were taken from the media and analyzed in accordance to the EPA analytic techniques. The result showed that removal of TPH in the soil using fungus *A. niger* was rather achieved in the above incubation periods. The best results were achieved for the medium (I) with the reduction of TPH, from the initial value of 48.300 ppm to 44.630, 39.430 and 33.600 ppm within 30, 65 and 96 days, respectively.

Keywords: Total Petroleum Hydrocarbons (TPH), *Aspergillus niger*, Bioremedaiton, Contaminated soil.

Introduction

The petroleum industry is responsible for the production of high amounts of organic residues, as well as for the pollution of soils, rivers and seas (Lemos et al., 2002a). When petroleum hydrocarbons are released through a spill or leak into the environment, they migrate down through soils, becoming adsorbed to the soil particles until they reach groundwater where they will dissolve in water, float on the water surface or sink to the bottom of a water aquifer (Balba, 2003).

Total petroleum hydrocarbons (TPH) are a mixture of chemicals, but they are all made mainly from hydrogen and carbon, called hydrocarbons. Scientists divide TPH into groups of petroleum hydrocarbons that act alike in soil or water. These groups are called petroleum hydrocarbon fractions. Each fraction contains many individual chemicals. Some chemicals that may be found in TPH are hexane, jet fuels, mineral oils, benzene, toluene, xylenes, naphthalene, and fluorine, as well as other petroleum products and gasoline components. However, it is likely that samples of TPH will contain only some, or a mixture, of these chemicals. The International Agency for Research on Cancer (IARC) has determined that one TPH compound (benzene) is carcinogenic to humans. IARC has determined that other TPH compounds (benzo[a]pyrene and gasoline) are probably carcinogenic to humans. Most of the other TPH compounds are considered not to be classifiable by IARC (Buyukgungor and Gurel, 2009; Romanus et al., 2015).

Physical, chemical and biological technologies have been developed to remove hydrocarbon pollutants from soils and to restore environmental quality. Bioremediation has become an attractive alternative to physicochemical methods of remediation, where feasible (Schaefer et al.,

2005).

The potentiality of the microorganisms, pointed out in literature as agents of degradation of several compounds, indicates biological treatments as the most promising alternative to reduce the environmental impact caused by oil spills. It is known that the main microorganisms consuming petroleum hydrocarbons are bacteria and fungi (Lemos at al., 2002). Among microorganisms, fungi stand out as potentially petroleum degrading organisms, since they are able to consume hydrocarbons even in low concentrations of nitrogen sources, indicating that nutritional depletion might stimulate the production of their enzymatic systems. Microbial processes can reduce hydrocarbons concentrations in soil to level that no longer pose an unacceptable risk to the environment or to human health (Lemos et al., 2002b).

This study investigated the use of fungus *Aspergillus niger* to remove TPH from soil polluted with crude oil and obtained from Southeast Anatolia of Turkey, and accordingly the biodegradation of TPH in soil by *A. niger* biomass was investigated (Yang et al., 2009; Buyukgungor and Gurel, 2009).

Materials and Methods

Soil: Soil samples were collected in Batman, Southeast Anatolia of Turkey and were accidentally contaminated due to a crude oil spill. All debris was removed from soil samples and then was air dried for 24 hrs. The dried soil was sieved to a particle size of 2 mm. Characterization of their physical and chemical properties was performed before bioremediation (Table 1).

Microorganism-growth conditions and inoculum: A fungal strain of *A. niger* (RSHMB-04017) was routinely maintained on potato dextrose agar plates. *Aspergillus niger* was inoculated into a liquid media comprised of dextrose (20 $g.L^{-1}$), peptone (10 $g.L^{-1}$), and yeast extract (3 $g.L^{-1}$) in distilled deionized water. 100 mL of the medium was transferred into 250 mL conical flasks. The flasks were placed on a rotary shaker operating at 125 rpm. All culture work was conducted under aseptic conditions at room temperature.

A carbon free mineral salt media (MSM) was used to check growth and degradation as described by (Faryal et al., 2006). Composition of the media was KH_2PO_4 (13.6%), $(NH_4)_2SO_4$ (2.4%) and NaOH (2.5%) for solution A and $MgSO_4.7H_2O$ (8.0%), $FeSO_4.H_2O$ (0.2%) and HCl (4%) for solution B. Mineral salt media was prepared by mixing 50 mL solution A and 7 mL solution B in 1 litre of distilled water. Mineral salt of glucose (MSM-G) was prepared by adding 5 mM glucose.

Table 1. Properties of petroleum contaminated soil sample.

pH	8.91
EC (electrical conductivity) (µS/cm)	180
TPH (ppm)	48300
Total phosphate (ppm)	55
C:N:P ratio	280000:250:1
Moisture (%)	92
Potassium (ppm)	28.00

The conidia of *A. niger* was suspended in MSM-G as 10% (w/v) and homogenized in a sterilized blender, and used as inoculum (Faryal et al., 2006; Semple et al., 2001).

Biodegradation proses: The degradation of the polluted soil containing 48,300 ppm of total petroleum hydrocarbon (TPH) was carried out in 250 mL filtering flasks, each containing 50 g of soil sample. The biodegradation of TPH was applied to three different soil media, including (I) contain only *A. niger* in sterilized soil, (II) soil microorganisms + *A. niger*, and (III) only soil microorganisms. The mediums I and II were inoculated by adding 15 mL inoculum. The medium III was not inoculated and only added 15 mL MSM-G for nutrition soil microorganisms. No nutritional adjustment was done. Based on those data the carbon, nitrogen and phosphorus (C:N:P) ratio was calculated and the results was: 280000:250:1. The experiment was conducted at 30°C for 30, 65 and 96 days without pH correction (original pH was 5.0). At the beginning and at the end of experimental periods, the samples of soil were taken from the media, and analyzed based on EPA analytical techniques (EPA Method 418.1) (EPA, 2016).

EPA Method 418.1 are the widely used methods to determine TPH in soils. The mixture of sodium sulfate and silica gel was added to the samples to take out any excess water/moisture in the samples and then added freon to each glass tube in TPH Method 418.1. The samples were sonicated for 20 minutes and then cooled. Each sample was filtered through a prepared glass pipette with fluorosis and glass wool into the spectrophotometer cuvette and then was placed in the spectrophotometer to read its absorbance. Then, the final concentrations of TPH (ppm) were calculated.

Table 2. TPH concentrations through incubations periods.

Medium	Time (days)			
	0	30	65	96
	TPH concentrations (ppm)			
Medium I (Only *Aspergillus niger* in sterilized soil)	48300	44630	39430	33600
Medium II (Soil microorganisms- *Aspergillus niger*)	48300	46220	42890	36510
Medium III (Only soil microorganisms)	48300	47930	45640	44460

Table 3. Regression equations and R2 values for the three integrated kinetic models.

Medium	Kinetic models		
	Zero-order	First-order	Second-order
Medium 1	y=-152.69x+48781 R^2=0.9917	y=-0.038x+10.803 R^2=0.9798	$y=10^{-7}x+2.10^{-5}$ R^2=0.9598
Medium 2	y=-119.77x+49199 R^2=0.9400	y=-0.0028x+10.807 R^2=0.9019	$y=7.10^{-8}x+2.10^{-5}$ R^2=0.9232
Medium 3	y=-43.02x+48637 R^2=0.9511	y=-0.0009x+10.787 R^2=0.9651	$y=3.10^{-8}x+2.10^{-5}$ R^2=0.9992

Kinetics: The kinetics of TPH removal was evaluated by the integral method, using zero-order (eq.1), first-order (eq.2) and second-order (eq.3) integrated kinetics model for three medium (Gheju, 2011; Ekperusi and Aigbodion 2015);

$$C_{TPH} = C^0_{TPH} - k.t \quad (1)$$

$$\ln C_{TPH} = \ln C^0_{TPH} - k.t \quad (2)$$

$$\frac{1}{C_{TPH}} = \frac{1}{C^0_{TPH}} + k.t \quad (3)$$

Where C_{TPH} is the TPH concentration at time t (ppm). C^0_{TPH} is the initial TPH concentration (ppm), k is the removal rate coefficient, and t is the time (days).

By plotting C_{TPH} (zero-order), ln C_{TPH} (first-order) and 1/ C_{TPH} (second-order) vs. time using the experimental data, a straight line should be obtained; the line equation was calculated by regression analysis. The conformity between experimental data and the kinetic model was expressed by the correlation coefficient R^2; the model that successfully describes the kinetics of the TPH removal is the one that has the highest R^2 value.

Results and Discussion

Table 2 shows the reduction of TPH obtained through the analysis of TPH by extraction of the initial and final samples. The best results were achieved for the medium (I) with the reduction of TPH, from the initial value of 48.300 ppm to 44.630, 39.430 and 33.600 ppm within 30, 65 and 96 days respectively.

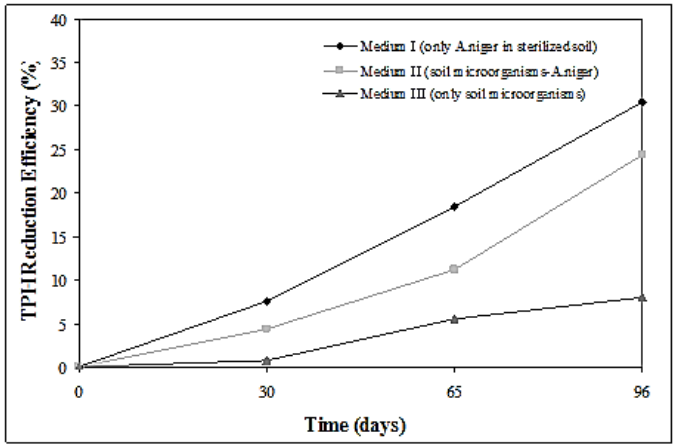

Figure 1. TPH removal efficiency (%) of *Aspergillus niger* from soil polluted with crude oil.

Figure 1 represents the removal TPH efficiency obtained with the addition of *A. niger* to the polluted soil and monitored in a period of 96 days. The removal TPH affinity order is as follows: medium I (30%) > medium II (25%)> medium III (8%). More than 30 percent degradation was achieved in less than 100 days.

Subsequently, the observed rate coefficients were deduced from the equation with highest R^2. The regression equations and R^2 values for the three integrated kinetic models, calculated at Medium 1, Medium 2 and Medium 3, are presented in Table 3.

The results also show that the removal of TPH can be approximated with the zero-order integrated kinetics model for Medium 1. The smallest value of correlation

coefficient was >0.95 for Medium 1 (Table 3).

Conclusion

Bioremediation is a promising technology for the treatment of a wide range of contaminants in soil and groundwater. The method is cost-effective, particularly for dealing with petroleum hydrocarbon contamination, and can be easily integrated with other remedial technologies. The degradation rate of hydrocarbons by these methods is dependent on the type of contaminants, metabolic capabilities of the indigenous microbial population, and also on predominant environmental factors (Buyukgungor and Gurel, 2009; Romanus et al., 2015).

This study investigated the use of fungus *A. niger* to remove TPH (Total Petroleum Hydrocarbons) from soil polluted with crude oil from Southeast Anatolia of Turkey. The results revealed that removal of TPH in the soil *A. niger* was rather achieved in the above incubation periods. The use of *A. niger* significantly improved the rate of petroleum hydrocarbon biodegradation in polluted soil. The results also showed a good coherence among regression equations and R^2 values.

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