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Bioremediation of Contaminated Soils with High Concentrations of Hydrocarbons (Diesel and PAH) using Three Biostimulants

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Abstract

The objective of the study was to carry out an experimental bioremediation project with soil from an industrial site contaminated with hydrocarbons by diesel (MFH) tank spills and Polyaromatic (PAH) generated in the various processes. The soil contains high proportions of fines that are more difficult to degrade.

Three experiments were performed, the first consisted of placing contaminated soil in 12 aluminium trays and adding two types of nutrients in two concentrations: blood meal and compost. The aerobic bacteria content (CFU/g) was monitored throughout the experiment and the water content was kept constant with daily aeration.

The results showed a very good removal efficiency MFH (70%) despite its high concentrations and the adsorption promoted by the content of fines in the soil. The best results were obtained with blood meal and compost. The degradation of PAH was very low with only bioremediation, so an additional experiment was carried out where the soil was first washed with surfactant and subsequently aereated. PAH decreased 70%.

Keywords: *Bioremediation; Blood Meal; Contaminated Soil; Hydrocarbons Degradation; Medium Fraction Hydrocarbons (MFH); surfactant*

Introduction

Bioremediation of soils is currently a relevant issue, because it implies a process in which organic contaminants in the subsoil are biodegraded and can become mineralized so that eventually they become non-toxic compounds. The contaminant does not enter another physical state because it is degraded.

Bioremediation is aimed at maintaining the maximal possible growth of microorganisms until the carbon source (organic contaminant) decreases and, consequently, the microbial population decreases [1,2]. Physical and chemical factors are needed for an efficient bioremediation process; including water, temperature, pH, oxygen, major and minor nutrients.

Water: Water content is one of the most important factors for degradation, since water constitutes 80 to 90% of the weight in the molecular composition of bacterial cells and is the main nutrient [3].

pH: The intracellular pH value lies between 6.5 and 7.5, hence this is the required pH range needed for optimal microorganisms' growth.

Temperature: The chemical and enzymatic reactions of the cell increase concomitantly with increasing temperature. There are: a minimal temperature for each organism, below which no growth occurs, an optimal temperature at which growth is faster, and a maximal temperature above which no more growth occurs. The temperature range considered optimal for heterotrophic aerobic bacteria is between 20°C and 35°C [4].

Oxygen: Oxygen is the electron acceptor most used by microorganisms to degrade organic compounds in an aerobic environment. If the oxygen content of the soil is below 2 mg/l, conditions are favorable for an anaerobic environment.

Nutrients: The solid portion of the bacterial cell is constituted by carbon, nitrogen, hydrogen, phosphorus, and, to a smaller extent, potassium, calcium, magnesium, chlorides, iron, and others. The main component (50%) is carbon. The contaminant to be degraded must contain this element. Oxygen, with 20%, is the second most abundant element in the cell. Oxygen is needed for new cells and as electron acceptor, hence, it is necessary to count upon large amounts of oxygen for biological degradation. The other major nutrients required by microorganisms are nitrogen and phosphorus. The three main nitrogen sources in microorganisms are proteins, cell wall constituents, and nucleic acids. Phosphorus, in the form of phosphates, is used by microorganisms to synthesize phospholipids and nucleic acids [5].

Factors that might limit the activity of microorganisms are low temperatures, very low o very high pH values, and chemical agents, such as heavy metals, halogens, organic and oxidizing contaminants. The main techniques applied to bioremediation are: in situ and ex situ bioremediation, bio piles, land farming, phytoremediation, bio augmentation, bioventing. Bioremediation technique consists in forming mounds with the contaminated soil and stimulating the microbial communities through aeration and/or by adding nutrients and water. The increment in microbial activity is directly proportional to the reduction in total Petroleum Hydrocarbons (HTP) concentrations. Bioremediation aimed at reducing the concentration of hydrocarbons that are adsorbed in contaminated soils by means of biodegradation [6-7]. Bio piles is the most commonly used technique to treat soils contaminated with petroleum hydrocarbons [8-12]. Bioremediation has proven to be one of the most used techniques in the world [13-15]. Microbiological activity can be stimulated by supplying oxygen, through aeration, and water and nutrients, such as nitrogen and phosphorus.

The number of heterotrophic bacteries are the main parameter to know if degradation will be possible. The minimum of CFU/g for degradation to occur must be equal or higher than 103.

Efficiency of a bioremediation depends on several parameters, which are grouped in three categories [12] these are:

- Soil characteristics.
- Characteristics of the contaminants.
- Weather conditions.

The type of soil is very important because water, nutrients, and air must be able to migrate with some ease through the soil pores to allow microorganisms to accomplish degradation. Texture of the soil influences soil permeability and water content. Highly permeable soils are the most easily aerated and, therefore, are the most adequate to be used for bioremediation. Bioremediation ex situ must be made on an impermeable base to reduce the possible migration of lixiviates towards the subsoil. In addition, it is necessary a source of air through a compressor and a network of perforated tubes or via a mechanical truck which mix the soil to supply air to bacteria. The objective of this work was to clean soil with high content of fines contaminated with very high concentration of hydrocarbons by means of bioremediation. The contaminated site comes from an old industry complex which worked with diesel and PAH from chemical processes. The contaminated area is around 90,000 m^2 and an approximated volume of 200,000 m^3. The total area is 200 ha.

Due to the difficult to degrade high concentrations of hydrocarbons in fine soils it was decided to make an experimentation on laboratory with soil of the contaminated site and test different kind of nutrients: blood meal, compost and urea [16-19].

Blood meal is a dry, inert powder made from blood, used as a high-nitrogen organic fertilizer and a high protein animal feed. N = 13.25%, P = 1.0%, K = 0.6%. It is one of the highest non-synthetic sources of nitrogen. It usually comes from cattle or hogs as a slaughterhouse by-product [20].

The used compost comes from the trees of the site which has more than 50 ha of green areas.

Materials and Methods

About 200 kg of soil were removed from the contaminated site between 0.5 and 3.5 m deep and packed and translated to the laboratory of the Institute of Engineering at National University of Mexico (UNAM) where experimentation was conducted. The soil was characterized and its main parameters (pH, porosity, organic matter content, soil granulometry, water content, content of heterotrophic bacteria) were determined (Table 1).

Parameter	
pH	8.03
Electric conductivity (EC) (dS/m)	1.19
Organic matter (%)	5.87
Real density (g/cm^3)	2.39
Aparent density (g/cm^3)	1.40
Porosity	0.41
Field capacity (%)	42
Aerobic bacterial account (UFC/g)	700
N (mg kg^{-1})	46.65
P (mg kg^{-1})	4.03

Table 1: *Results of soil characterization.*

Hydrocarbons from diesel (FMH) and six polyaromatics (PAH) contained in the soil were analyzed: benzo(a)anthracene, benzo(a) pyrene, Benzo(b)phluoranthene, Benzo (k) phluoranthene, Dibenzo (a,h) anthracene, Indene(1,2,3) pyrene . For blood meal content of organic matter OM, pH, EC, N and P were analyzed. For compost, OM, and OC.

For the first experiment, in 12 aluminum trays 500 g of soil were added in each, four only with the contaminated soil to serve as blanks, four with blood meal with 25 and 50 g and four with compost and two different comtents 10 and 20 g (Table 2).

Every week presence of heterotrophic bacteries (CFU) were determined since first day and every 2 weeks the Fraction Medium Hidrocarbons (FMH) and PAH were analyzed. Every day the soil of 12 trays were aireated and every week water was added to maintain the same field capacity. Because the aerobia bacteria feeds from

Sample	Blood meal (BM)	Sample	Compost (C)
T1	Soil	T7	Soil
T2	Soil	T8	Soil
T3	Soil + 25 g BM	T9	Soil + 10 g C
T4	Soil + 25 g BM	T10	Soil + 10 g C
T5	Soil + 50 g BM	T11	Soil + 20 g C
T6	Soil + 50 g BM	C12	Soil + 20 g C

Table 2: *Setting of the experiment.*

the carbon of the hydrocarbons and the nutrients added, these also decrease and for this reason at the 45 days of starting the experiment nutrients were added in proportion to the remaining soil. The quantification of FMH is performed by Gas chromatography/Mass Spectrometry (GC/MS) on an Agilent 6890 gas chromatograph with selective mass detector, Agilent 5973, with Initial temperature: 80°C Maximum temperature: 350°C

In the following experiment, urea and triple phosphate were tested as the third bio stimulant to have the C: N: P ratio at exactly 100: 10: 1. For this experiment, three trays were placed where, unlike the previous experiment, the proportion of bio stimulants added to the soil complied with the C: N: P ratio of 100: 10: 1. To complete the amount of phosphorus needed, a commercial fertilizer was used, which is made up of 46% of this nutrient, and in addition, urea was used to complete the nitrogen deficit.

The quantification of aerobic bacteria in soil and blood meal was determined (Tables 3 and 4). The bacterial count in soils is carried out when it is necessary to investigate the content of viable microorganisms and the commonly used is the plate counting technique [21,22]. The variety of species and types differentiable by their nutritional needs, temperature required for growth, available oxygen, etc., make the number of colonies of bacteria counted constitute an estimate of the figure actually present. The plate count technique of aerobic bacteria in soil is based and adapted in the official norms NOM-092-SSA1-1994 [23], which establish the method to estimate the amount of viable microorganisms present, as well as its preparation and dilution of the samples, respectively. An HPLC equipment was used for the determination of PAHs, according to the NMX-AA-146-SCFI-2008 [24].

Sample	Week 1	Week 2	Week 3	Week 4
T1, T2	0.062	0.042	0.057	0.79
T3, T4	2.1	52.5	75	91
T5, T6	0.4	13.3.	8.75	80
Each value is x10 +6				

Table 3: *Colony forming units in blood meal (CFU/ g).*

Sample	Week 1	Week 2	Week 3 Week 4
T7, T8	0.02	0.95	1.0 0.45
T9, T10	0.045	0.95	2.40 2.31
T11, T12	0.03	0.42	1.5 3.55
Each value is x10 +6			

Table 4: *Colony forming units in compost (CFU/g).*

The experiment to test the degradation of PAHs consisted of adding 2 kg of the contaminated soil in a mixer and 500 ml of water with a surfactant (Tween 80) concentration of 50 ml/l were added. The mixing lasted 8 hours for two days. Subsequently, the water was decanted and the soil was allowed to dry for 24 hours. The soil was placed in a try with a system of 4 perforated ¼" tubes to which air was supplied for 24 hours. The system was covered to prevent the escape of vapours.

Results and Discussion

Table 1 shows the results of the characterization of the contaminated soil. According to NOM-021-SEMARNAT-2000 [25] is classified as a soil with a high content of organic matter, which in general is inconvenient for bioremediation due to the adsorption of pollutants that makes it more difficult to bioavailability. The other parameters are suitable for the technique to be effective, and it was considered that the addition of nutrients will be more effective the technique. Table 2 shows the experiment arrangement with 12 trays with contaminated soil and the addition of two nutrients with two concentrations.

The initial concentration of FMH in soil was 26,000 mg/kg and more than 100 mg/kg for PAH: benzo(a)anthracene (138), benzo(a) pyrene (137), Benzo(b)phluoranthene (222), Benzo(k)phluoranthene (59), Dibenzo (a, h) anthracene (41), Indene (1,2,3) pyrene (134). The permissible limit for each parameter is based on NOM-138.SEMARNAT/SSA1-2012 [26].

Table 3 shows the monitoring of aerobic bacteria whose count shows the importance of the addition of nutrients since the first week of the experiment the number of aerobic bacteria increased more than 33 times for blood meal tests for concentration less. For the concentration of 50 g in the first week it only increased 1.3 times. For week 4 the concentration of aerobic bacteria increased more than 1000 times for the two concentrations. Even when the number of degrading bacteria is unknown, it is considered that there is an optimistic possibility for biodegradation to take place.

In week 4 the increase was 145 times for the lowest concentration and 177 for the largest (Table 4).

Figures 1, 2 and 3 show the growth of bacteria in the presence of blood meal, compost and urea. In all three cases the growth is very favorable although the highest growth is shown for blood meal.

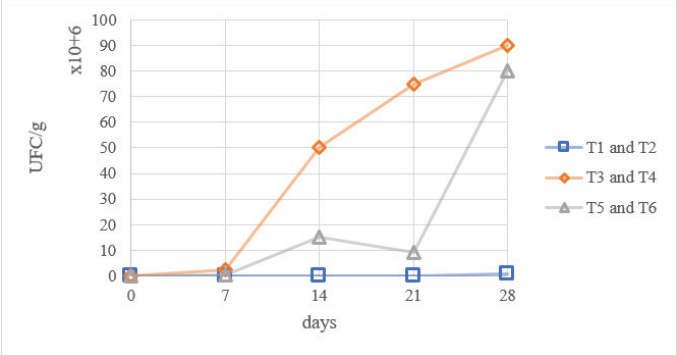

Figure 1: *Bacteria growth in soil with blood meal.*

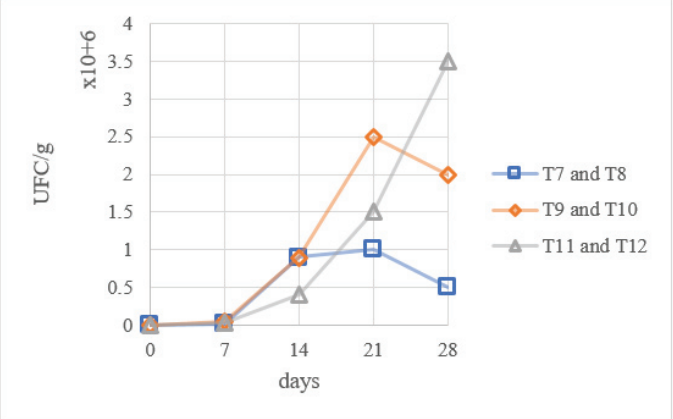

Figure 2: *Bacteria growth in soil with compost.*

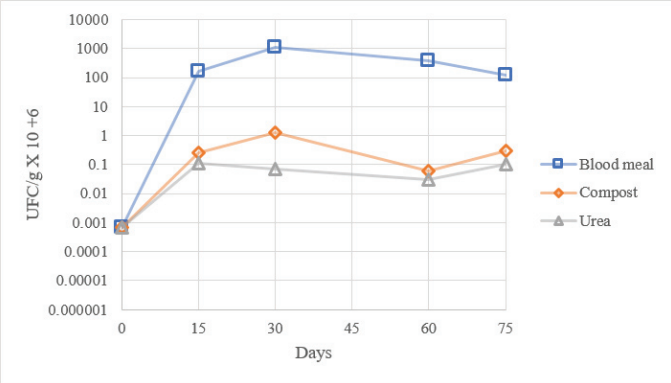

Figure 3: *Bacteria growth in soil with blood meal, compost and urea.*

For week 6 a removal efficiency of between 66 and 70% was obtained for the lowest concentration of blood meal (Figure 4). For the experiment with compost, removal efficiencies between 65% and 67% were obtained for the lowest concentration (Figure 5). Both results were obtained 45 days after the experiment. Although the permissible concentration (PL) was not reached in any case according to NOM 138 (5000 mg/kg) it is considered a good removal efficiency given the characteristics of the soil and the high concentrations, for the time of experimentation (Tables 5-8).

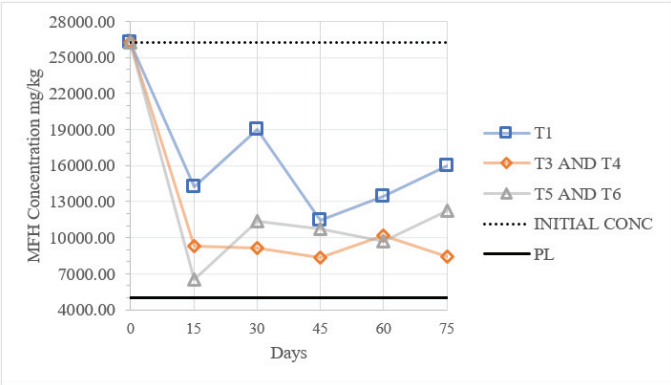

Figure 4: *Degradation of MFH in experiment with blood meal.*

Sample	Week 2	Week 4	Week 6
Initial concentration of MFH: 26000 mg/kg			
T1	13963	19363	9868
T2	14565	18721	13009
T3	8807	8743	7808
T4	9844	9550	8943
T5	5991	10610	10489
T6	7096	12170	11037

Table 5: *Degradation of MFH using blood meal.*

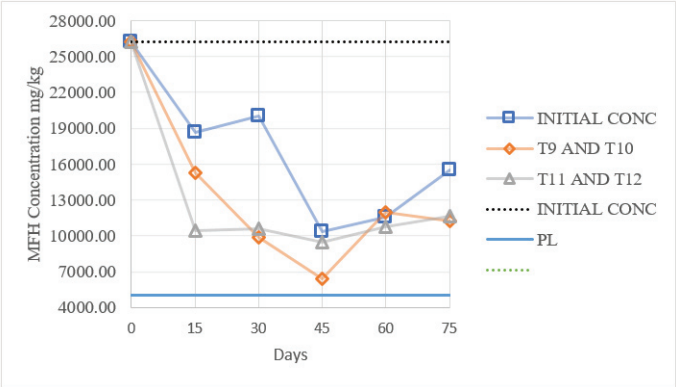

Figure 5: *Degradation of MFH in experiment with compost.*

Sample	Week 2	Week 4	Week 6
Initial concentration of MFH: 26000 mg/kg			
T7	16243	18619	15669
T8	21041	21355	11401
T9	15683	11273	9083
T10	14800	8510	8584
T11	11678	10429	9301
T12	9249	10753	9896

Table 6: *Degradation of MFH using compost.*

Sample	Week 2	Week 4	Week 8	Week 10
Initial concentration of MFH: 19594 mg/kg				
Blood meal	15736	11678	8945	9864
Urea	14965	12002	11183	10993
Compost	33579	19019	15510	8418

Table 7: *Degradation of MFH using blood meal, compost and urea.*

The results of the soil with urea were as expected since the population of bacteria did not grow as much as with the other nutrients. However, although urea has a population of bacteria up to a thousand times less than blood meal, the results were not in this proportion since a degradation between 44 and 49% was obtained. This may be due to a better amount of degrading bacteria in this nutrient (Figure 6).

PAH	Sample 1	Sample 2	Initial	Removal
	mg/kg		%	
Be Benzo(a)anthracene	66	48	138	52 - 65
Benzo(a)pyrene	55	35	137	60 - 74
Benzo(b)phluoranthene	99	54	222	55 - 76
Benzo(k)phluoranthene	25	16	59	58 - 73
Dibenzo(a,h) anthracene	16	12	41	61 - 71
Indene(1,2,3) pyrene	50	37	134	63 - 72

Table 8: *Degradation of PAH using surfactant and aeration.*

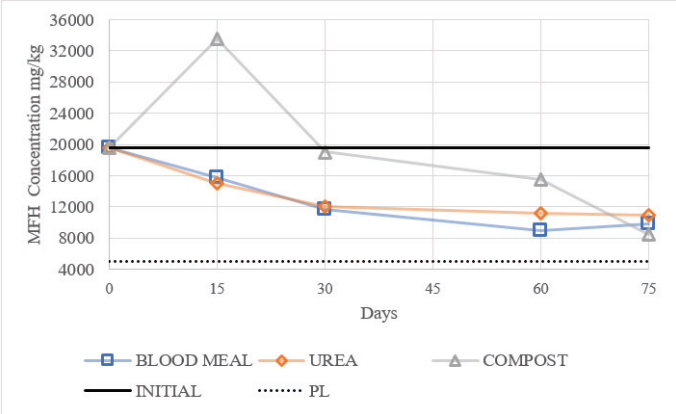

Figure 6: *Degradation of MFH in experiment with blood meal, urea and compost.*

Figures 4, 5 and 6 show curves with ascents and descents, this is normal since the soil was not screened or homogenized with the idea of bioremediation under real conditions; Therefore, what is observed is the tendency to reduce concentrations with both flood meal and compost as is cited by Herrera [27-28].

Sayara et al. [29] and Namkoong [30] mention that after 30 days of using compost as nutrient, the removal of PAHs was 89% and total oil hydrocarbons 98% respectively; however, in the experiments of the present work after 75 days of bioremediation there was no decrease in PAHs and as for MFH similar percentage of removal is not achieved. This is most likely due to the organic matter content of the soils used by these authors, due to the differences in soil-compost ratios used in this work and the different characteristics of the compost used by each author. Wang et al. [31] indicate that after 150 days the soil with blood meal and weekly aeration also shows a low removal of PAH (26%) and in this work the removal is less than 20%. This shows the difficulty of degradation of polyaromatic compounds that are also considered recalcitrant.

The content of fine materials in the soil and high organic matter adsorbs more hydrocarbons with complex chemical structure such as PAHs. For this reason, it was decided to carry out another experiment with soil washing. The non-ionic surfactant used has great capacity to desorb the organic compounds and this was demonstrated in the experiment. Subsequent aeration promotes de degradation of PAHs. With this experiment excellent results were obtained in one week with removal efficiencies of the order of 70%. It can be considered that both the use of blood meal and compost show a good tendency to degradation of MFH in a short time of experimentation. However, for the selection of the nutrient in an on-site pilot test and subsequently application in the site will not only depend on the results obtained in this work but on the economic factor.

The advantage of the compost over the blood meal is that it is close to the site and the company has the necessary infrastructure to transport the amount needed for bioremediation, so economically the compost would be a better option than blood meal.

Conclusion

It is considered that the results for hydrocarbons derived from diesel were very good since even when the permissible levels were not obtained, the removal efficiencies were more than 65% with respect to the initial concentrations of between 45 and 75 days

This project consisted of testing possible solutions to apply in the remediation of the contaminated site so the results indicated that the best option would be to test an experiment in the field with a volume of approximately 300 m^3 by first applying a soil washing and then bioremediation with blood meal or with compost.

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