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Restoring A Coastal Soil Contaminated with Weathered Hydrocarbons through Bioestimulation, Bioventing and Bioagmented Technologies at Minimal Cost

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Abstract: The degradation of weathered hydrocarbons was studied in a laboratory level experimental design. Samples of contaminated soil were placed in 1 liter glass containers. The contaminated soil had an initial hydrocarbon concentration of 55,325±50 mg HF/kg dry soil. Two series of experiments were performed under the following conditions: 1) additions of Bioaugmented microbial consortium + injected air flow + moisture + optimized minimal medium (MM), 2) additions of Bioaugmented microbial consortium + injected air flow + moisture +Bushnell Haas enriched medium (BH). In both series the following operating parameters were varied: % moisture, airflow and nutrient level, each evaluated in low and high levels following a Plackett-Burman experiment design. The response variables were: HF (Heavy fraction)/kg dry soil content, the FTIR analysis, percentage organic matter, pH and electrical conductivity (mS/cm) of the soil. The results obtained showed a maximal removal of 41.3% HF/kg dry soil after 14 days of biodegradation with the following conditions: 40% moisture content, 80 L air/h with added nutrients. Alteration of some physicochemical characteristics of the soil were also observed at the end of the tests. The treatment that showed the high removal change from 5.5 (acidic) to 7.8±0.5 (neutral), and the EC (mS/cm) increased from 0.4±0.1 to 0.9±0.1 in the control. The experimental results indicated optimal operating parameters to enhance the bioavailability the weathered hydrocarbon contaminated soils at minimum cost.

Key words: bioventing, biostimulation, bioremediation, hydrocarbon recalcitrance

1. Introduction

The weathered hydrocarbons studied here were chiefly heavy and medium fractions of oil of high molecular weight, commonly as hydrocarbon chains containing up to 25 carbon atoms. Weathering refers to the result of biological, chemical and physical processes that can affect the type of hydrocarbons that remain in a soil. Weathering examples include volatilization, photooxidation, biodegradation, emulsification and sedimentation which all increase hydrocarbon recalcitrance.

The implementation of a single bioremediation technology does not produce complete weathered hydrocarbon degradation due to their chemical complexity, however, implementation of a single technology works if it is designed to degrade individual pollutants [1]. Therefore it is necessary to couple various remediation technologies to treat contaminated soil efficiently, particularly when there are highly recalcitrant hydrocarbons or when there are high concentrations of metals [2]. This study proposes an alternative method for contaminated soil remediation of weathered hydrocarbons coupling technologies including bioventing, biostimulation and bioaugmentation. In testing the team evaluated the effects of the following parameters: airflow, concentration-type nutrients and moisture content in

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order to degrade the maximum amount of weathered hydrocarbons at minimum cost.

2. Material and Methods

2.1 Soil

Contaminated soil was collected from a site where oil waste has been deposited for decades. The source is located in the area of an industrial complex with high petrochemical activity in the Agua Dulce city, located in the State of Veracruz, México (18°09'N and 94°08'W and 20 meters above sea level). Soil samples were taken from the upper 15 cm depth and analyzed following the specifications of the NOM-138-SEMARNAT/SSA1-2012. Analysis of the organic matter by the ignition method was used to quantify a total content of organic and inorganic carbon [3]. The organic matter content was 18.8%. The Analysis of physicochemical properties was performed based on the NOM-021-RECNAT-2000 [4] methods. The electrical conductivity (EC) was determined by the AS-18-2000 method adding deionized water followed by stirring and then samples were allowed to stand, the EC of the supernatant was measured using a HACH TDS conductivity meter. The pH was measured using a potentiometer model OAKTON pH/Conductivity/TDS/°C/°F meter with a soil-water ratio of 1:2. Moisture content was measured from a 0.5 g sample of contaminated soil using CIATEC's Sartorius MA 35 meter. The determination of HF/kg dry soil was realized following the specifications of the NMX-AA-145-CFIDS-2000 standard (the minimum quantifiable concentration by the gravimetric method is 3000 mg of HF/kg of dry soil). Results showed an initial hydrocarbon concentration of 55325±50 mg HF/kg dry soil, with an EC of 0.11 mS/cm and pH of 5.5.

It is known that there is a high sorption of weathered hydrocarbons when the clay content is greater than 38%. The granulometric structure of the contaminated soil under study was 54% clay, 25% silt and 21% sand. The absorbance intensity of characteristic heavy hydrocarbons band was determined by infrared spectrophotometry (FTIR) following the protocol of [5]. Analyses by infrared spectrophotometry of the contaminated soil produced qualitative information on hydrocarbon content at the beginning and the end of the 14 day biodegradation test period.

Quantification of CFU/g dry soil was conducted by the streak plate technique using a round loop which was sterilized directly to the flame. Serial dilutions were carried out using a dilution factor in the order of 10-1 to 10-8. The total number of degrading bacteria was estimated with a solid medium of Bioxon trypticase soy agar (TSA) BD at 35°C, selective for bacteria [6]. It was found that the initial bacterial density before bioaugmentation with special culture was 1.7×10^5 CFU/g dry soil while the final value for the best treatment was of 10.62×10^5 CFU/g dry soil.

2.2 Bioaugmentation

Prior to this work, a microbial consortium was obtained with the potential to degrade the heavy fraction in the Maya oil. The bioaugmented inoculum contains the following bacterial species: *Flavobacterium salisperosum, Flavobacterium terrae, Flavobacterium salisperosum, Pseudomonas citronellolis, Pseudomonas delhiensis, Pseudomonas knackmussisi, Ochrobacterium intermedium, Ochrobactrum cytisi, Ochrobactrum anthrop and Ochrobactru tritici,* each one a percentage of identity of 98%.

2.3 Biostimulation

The nutritional conditions were optimized so that the microorganisms had the necessary minimum concentration to carry out their metabolic processes efficiently. This media is here denoted 'minimal medium' (MM) and contains the following salts: (gL^{-1}) 0.001 NaCl, 0.006 $MgSO_4{\bullet}7H_2O$, 0.002 $CaCl_2$, $1(NH_4)_2SO_4$, $1KH_2PO_4$, $1FeCl_3{\bullet}6H_2O$, 0.001 $(NH_4)_2PO_4$, 0.25 NH_4Cl, 1.065 $NaHPO_4$, 0.1 $FeSO_4$, 0.5 $NH4NO_3$. In order to compare the efficiency of biodegradation of weathered hydrocarbon an enriched medium: Bushnell-Haas (BH) was also employed. This medium contains the following salts: (gL^{-1}): 60 g KH_2PO_4, 60 g $(NH_4)_2HPO_4$, 60 g KNO_3, 3 g $FeCl_3$, 1.2 g $CaCl_2$, 12 g of $MgSO_4.7H_2O$. Two levels of medium concentration were tested: adding 57.6 g BH/kg dry soil and 0 g BH/kg dry soil for low and high level respectively while was adding 1.47 g MM/kg dry soil and 0 g MM/kg dry soil for low and high level respectively.

2.4 Bioventing

The application of controlled air flow has been shown to increase native microbial activity in a soil. Earlier research has shown that application of air and moisture under optimal conditions impacted the ability of microorganisms to perform soil degradation [7]. During testing then, two levels of air flow/hour were evaluated: 80 L air/h and 210 L air/h. The air flowed directly into the glass containers containing contaminated soil. When employed, flow was constant and aeration was performed for 24-hour periods each separated by periods of 48 hours when the pumps were turned off. During this quiescent time microbial activity occurs and after 48 hours the jars were uncapped for 15 minutes to avoid anaerobic conditions setting in.

2.5 Configuring Experiments: Bioventing, Biostimulation and Bioaugmentation

The designed operating parameters: moisture content, air flow (L/h) and nutrient content (grams/liter) were employed in two experiments studies, both sets of studies were carried out using the same conditions of moisture content, air injection and the addition of 1% bioaugmented microbial consortium at a level of 30 mL of inoculum solution to 300 g of dry contaminated soil. The two parallel tests were designated based on the enrichment medium used, thus the experiments were identified as: BH and MM, and note that during testing half of the experimental treatments were performed with no nutrient additions. Therefore, to perform a complete experimental replicate a total of 12 experiments were evaluated over the 14 day test periods. The biodegradation capacity during both experiments series (BH and MM) were compared to a control sample test run at 30% moisture and 80 L air /h, without an addition of the microbial consortium or nutrient addition. The conditions of the control were established by considering optimized enriched media together with adapted microbial consortia effects on highly recalcitrant hydrocarbons to achieve biodegradation of hydrocarbons in a coastal soil [7]. All experiments were run at temperatures ranging between 20°C and 28°C. Fig. 1 shows the operational setup and the response variables involved in the biodegradation process. The response variables measurements were considered a direct way to determine biodegradation capacity of the weathered hydrocarbon samples and were evaluated before and at the end of the 14 day biodegradation test period.

The biodegradation capacity of weathered hydrocarbons was assessed after 14 days using a Plackett-Burman fractional factorial design [8]. The tests were run in accordance with the levels suggested in Table 1. Thus treatment condition 1, 3, 6 and 7 were replicated. Once using the MM medium addition and once using the BH medium addition. The result of each

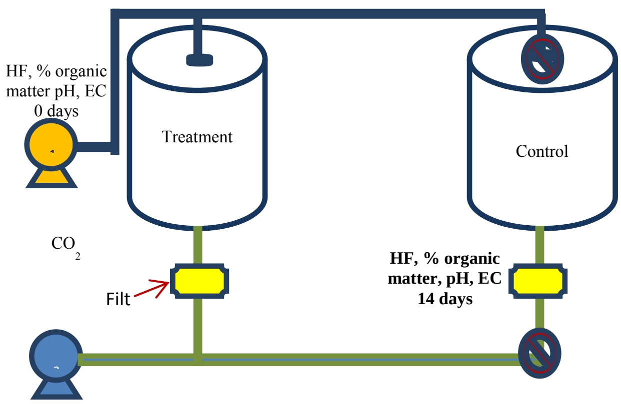

Fig. 1 Input and output variables of the coupled biostimulation, bioventing and bioaugmentation testing setup.

Table 1 Levels of operating factors for hydrocarbon biodegradation of the two experiments MM and BH.

Test	Treatment	% Moisture	Air injection level (L/h)	Nutrient Level (g/L)*
1	C1B1	30	210	MM/BH
2	C2B1	40	80	0
3	C1B2	30	80	MM/BH
4	C2B2	40	210	0
5	C1B3	30	210	0
6	C2B3	40	80	MM/BH
7	C1B4	40	210	MM/BH
8	C2B4	30	80	0

* MM: Minimum media, BH: Bushnell Haas media

test condition was qualified based on the level of weathered hydrocarbon degradation observed.

A moistened 300 g sample of contaminated soil, previously sieved with a mesh of 2 mm, with an added microbial consortium and a 30 mL vial of NaOH (introduced to capture the CO_2 produced by biomass digestion), were placed in the test chambers shown in Fig. 1. To avoid the admission of dust or microorganisms a filter fiber: Whatman Sigma Aldhrich of 0.45 μm was connected between the glass container and the pump hose. The glass containers were sealed and opened every 72 hours to prevent anaerobiosis during the 48 hours without active ventilation when most microbial activity occurs. At the end of 14 days, soil samples were taken from the glass containers, dried at room temperature and used for the response analyzes.

3. Results and Discussion

3.1 Bioestimulation, Bioaugmentation and Bioventing Coupling

Table 2 shows the results obtained of heavy fraction content using physicochemical analysis of contaminated soil for both experiments employing BH and MM as mineral enrichment medium. Most of the experiments that used MM had greater removal of hydrocarbons compared to those using the enriched BH medium meanwhile C1B4 with MM achieved 43.2% removal level. Another important observations in treatments with MM were the pH values moving closer to neutrality, as well as the decrease in the percentage of organic matter which is associated with the diminishing of the content of hydrocarbons. Table 2 shows that all treatments which employed BH as enriched medium tested at more than 13% organic matter while treatments that used MM testing at less than 13% organic matter, this reduced concentration could be considered an indirect measurement of quantified loss of organic compounds as the original weathered hydrocarbons, however, this result should be carried out with other analyses to validate this conclusion.

Table 2 Physicochemical analysis and heavy fraction contend of contaminated soil 14 days of biodegradation for treatment with nutrient addition and no nutrient addition.

	BH						MM					
Test With nutrients	mg HF/kg dry soil	% Removal	% Organic Matter	CFU × 10^5/g dry soil	EC (μS/cm)	pH	mg HF/kg dry soil	% Removal	% Organic Matter	CFU×10^5 /g dry soil	EC (μS/cm)	pH
C1B1	38400	27.9	15.58	5	0.32	8	36131	32.1	11.475	6.87	0.84	7.32
C1B2	40295	24.3	14.18	4.23	1.05	8.5	41071	22.8	12.26	11.75	1.01	7.42
C2B3	33165	37.7	13.25	4.17	0.89	7.9	41958	21.2	12.95	8.12	0.5	7.32
C1B4	32700	38.6	14.79	3.75	1.02	8.2	30214	43.2	12.48	10.62	0.41	7.8

On the other hand, CFU quantification represents bacterial growth under the limitation of hydrocarbons as a substrate [9]. Therefore in this study CFU measurements were carried out to measure bacterial growth and corresponding biodegradation of hydrocarbons at the beginning and at the ending of the experiment (zero hours and 14 days). According to these measurements all treatments with BH showed lower CFU/g dry soil content (between 3.75×10^5 CFU/g dry soil and 5×10^5 CFU/g dry soil) compared to treatments with MM who´s values were between of 6.87×10^5 CFU/g dry soil and 11.75×10^5 CFU/g dry soil. However it is important to understand that no species of bacteria that were quantified belonged to any known bacterial species with biodegradation potential. The team, therefore, suggests that further studies be carried out to identify additional types of bacteria with the capacity to biodegrade hydrocarbons. The highest bacterial density was obtained during test conditions C1B2 and C1B4 with MM at 11.75×10^5 CFU/g dry soil and 10.62×10^5 CFU/g dry soil respectively.

Table 3 presents results from the test treatments with no nutrients. It can clearly be seen that they indicate higher hydrocarbon content as well as the percentage organic content, electronic conductivity and pH than those treatments with added nutrients.

Table 3 Physicochemical analysis and heavy fraction contend of contaminated soil 14 days of biodegradation for treatment with no nutrient addition.

Test No nutrients	mg HF/kg dry soil	% Removal	% Organic Matter	CFU$\times10^5$/g dry soil	EC (μS/cm)	pH
C2B1	37211	30.0	14.875	9.37	0.455	7.855
C2B2	37337	29.8	13.17	1.1	0.525	7.655
C1B3	38639	27.4	12.795	6.96	0.55	7.755
C2B4	35662	33.0	13.14	2.66	0.49	7.65
Control	36060	32.2	18.12	0.006	0.9	8.2

The CFU content in the control the lowest value found in the entire experimental study suggesting that it is crucial to introduce an adapted microbial consortium, and the specific nutrients in order to make available microorganisms to consume weathered hydrocarbon.

3.1.1 Biodegradation of Heavy Fraction in Control Studies

The biodegradation of hydrocarbon during the control tests after 14 days of biodegradation (36446 mg HF/kg dry soil) was found. This evidence suggests that there is a certain capacity of the native microbiota to degrade weathered hydrocarbons (pretesting 55325±50 mg HF/kg dry soil to 36446 mg/kg dry soil) when aeration and moisture levels are regulated. The team concludes that optimum air flow with a suitable moisture content and nutrients can be expected to activate the capacity of native microorganisms and improve the metabolic processes of bioaugmented microorganisms to aid in the degradation of contaminates.

3.2 Identification of New Byproducts by FTIR Analysis

3.2.1 Identification of New Byproducts by FTIR Analysis

The intensity of several response band was observed at zero and 14 days of biodegradation compared the absorbance to the control (Fig. 3).

In the contaminated soil without any treatment an absorbance of 2.38 was found. FTIR spectra of treatment C1B4 with MM (40% moisture content +80 L air/h+ nutrients) is showed in Fig. 4 and a decrease of absorbance of the characteristic band of the heavy fraction (2918.6 cm^{-1}) was noted: from 2.38 to 1.47. Others bands indicate carbonates and nitrates, the spectra showing C-Cl and –C-N-C stretch.

The presence of these compounds in the final treated soil samples suggests the existence of a microbial species with different intermediates to develop products that benefit weathered hydrocarbons degradation.

Some authors have reported the appearance of chlorinated compounds during biodegradation processes. However, these authors have not defined the causes and origin of these elements in treated soils. Their presence may be related to the existence of inorganic compounds that are strongly adsorbed to the soil matrix, but which have been released into the free phase and could be identified after the bioremediation process is complete. Fig. 5 presents the infrared spectroscopy analysis of C1B4 treatment with BH as enriched media, note the higher hydrocarbon concentration after 14 days of biodegradation compared to the control. It this BH based test case the absorbance was 2.50 compared to the 2.38 of the control.

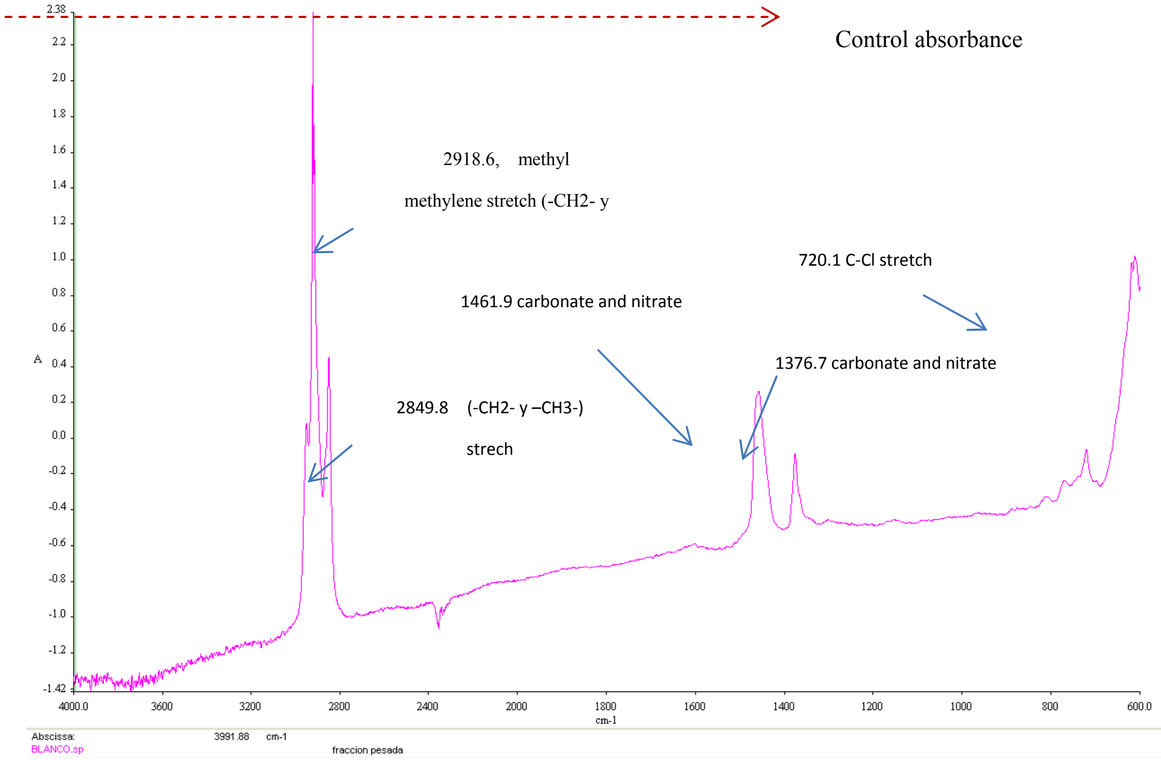

Fig. 3 Analysis HTPS infrared spectroscopy for control.

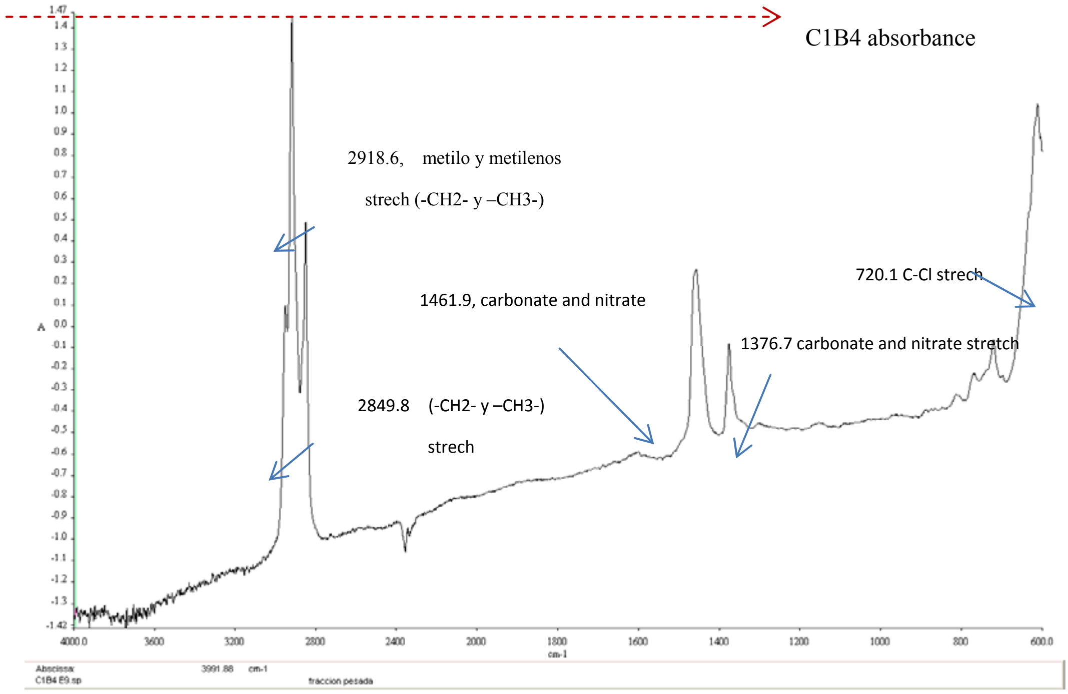

Fig. 4 Analysis FTIR for C1B4 with MM.

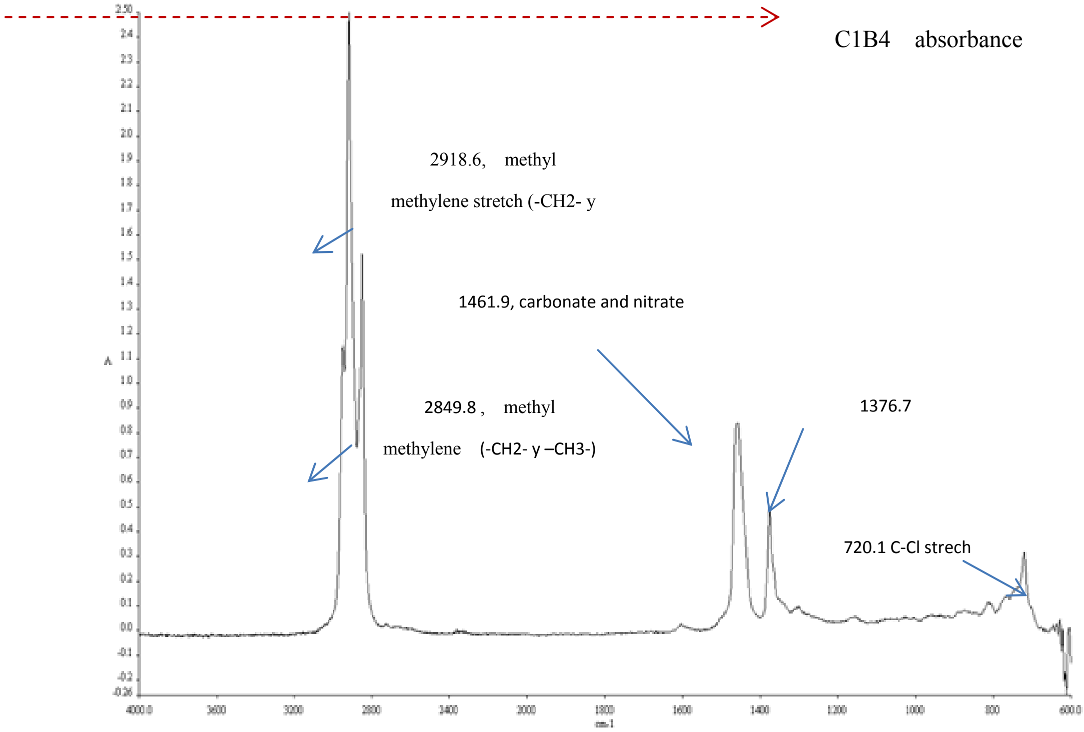

Fig. 5 FTIR analysis C1B4 with BH.

At times the soil conditions can lead to the production of more recalcitrant compounds which can be identified at the end of the process as more complex compounds than at the beginning of the experiment.

The production of specific enzymes by hidrocarbonoclastic microorganisms improves the generation of fatty acids and alcohols among other non-toxic compounds that contribute to recalcitrant hydrocarbons biotransformation. These byproducts influence desorption and solubilization of hydrocarbons and mass transfer through the soil matrix including those mineral salts may be embedded in the mineral fraction and can be consumed by microorganisms as an energy source. Thus the control of nutrients levels, such as nitrogen, can aid in the development of biosurfactants that promote the degradation of recalcitrant hydrocarbons [2]. The ideal environmental conditions in contaminated soil are reached if the appearance of a protozoa that impedes hydrocarbonoclastic bacteria growth is inhibited. In this study, the addition of adapted microorganisms to the heavy fraction of the contaminated soil did not appear to promote antagonistic effects against the native microorganisms in the soils.

The treatment using 40% moisture content showed greater hydrocarbon degradation, regardless of which or how much enriched medium was applied to the contaminated soil, indicating that slightly higher but controlled moisture content is a critical factor during degradation of weathered hydrocarbons [7]. The results suggest that the enriched medium (optimized MM) provided the minimum requirements necessary for microorganisms to carry out their metabolic processes to readily biotransform weathered hydrocarbons into simple organic molecules.

The success of the C1B4 treatment with 40% moisture content and 80 L air/h with the addition of nutrients indicates that one can expect significant biodegradation of contaminated soil only by adding the proper bacterium and using optimal moisture content and air flow. When operating conditions such as moisture content and air flow together with the necessary minimum amount of nutrients are established it is possible to achieve efficient metabolic activity [2] and therefore optimal biodegradation. In fact, as seen here, in some cases it may be possible for microorganisms to obtain the essential nutrients to carry out their metabolic processes from the mineral fraction or organic matter present in the contaminated soils without the addition of nutrients. In some cases the team found an inhibition of hydrocarbon biodegradation in tests with nutrient additions which was likely caused by mutagenic alterations to microorganisms. Studies reported by Popoviciu D. R. and Bercu R. (2014) [10], employing biostimulation process with microcosms to which were added inorganic nutrients including nitrogen and phosphorus, found inhibition reduction of polyaromatic hydrocarbons (PAHs) removal compared to treatment where nutrients were not added. This team offers further indications that it is crucial to optimize the nutritional conditions in order to allow the operational conditions to perform biodegradation efficiently considering both costs and time during soil remediation. Other studies performed by also reported that the biodegradation process was inhibited by adding some macronutrients. Again, then, it is critical to establish accurate levels of each nutrient for metabolic activity efficiency. This value will depend on the soil physicochemical characteristics and microbiota natures as well as pollutant origin.

4. Conclusions

Coupling biostimulation, bioaugmentation and bioventing increased the biodegradation capacity of weathered hydrocarbons present in the soil of Agua Dulce, Veracruz, Mexico. Control of operational conditions including moisture content, air flow and the concentration and type of nutrients allowed the team to find the best environmental conditions to maximized bioremediation. Treatment C1B4 with 40% moisture content, 80 L air/h and employing MM as enriched medium exhibited a removal of 43.2% mg HF/kg dry soil, compared to the control which obtained 32.2% mg HF/kg dry soil reduction. In some treatments with addition of high percent of moisture and air flow together with addition of adapted microbial consortia showed a lower biodegradation response.

This is associated with the investigations of some researches where the use of controlled air and moisture are enough to carry out metabolic processes efficiently by “native” microorganisms in a contaminated soil. However, this team showed that it is possible to increase the percentage of removal by an optimization of nutrient, air flow and moisture as exhibited here. The change on physicochemical parameters indicated a significant solubilization and desorption of hydrocarbon sorbed in the soil matrix. Generally it was observed that those treatments with lower air flow and moisture, with addition of MM mineral medium, had a better performance resulting in a low percentage organic matter and physicochemical properties like near-neutral pH.

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