Chapter 5

Laboratory-Scale Biodegradation of Fuel Oil No. 6 in Contaminated Soils by Autochthonous Bacteria

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Abstract

In order to evaluate the degradation of fuel oil no. 6 (FO6) in contaminated soil, laboratory-scale bioreactors were set up to study biostimulation, bioaugmentation, and natural attenuation processes. A solution of fertilizers was added in biostimulation and biouagmentation (0.03% N, 0.01% P). To the bioaugmentation process, an enrichment culture of indigenous hydrocarbon-degrading microorganisms was also added once a week. Total aerobic and hydrocarbon-degrading microorganisms were determined by plate count, and total petroleum hydrocarbon (TPH) concentration was determined gravimetrically (EPA method 9071b) every 15 days. After 1 year of study, degradation rate was higher for biostimulation (0.19 g TPH/day), followed by natural attenuation (0.18 g TPH/day) and bioaugmentation (0.16 g TPH/day). TPH showed a change in composition of hydrocarbons, attributed to microbiological activity. Microbial counts of hydrocarbon-degrading microorganisms were on the range of 4–6 log CFU/g soil. Preliminary bacterial identification corresponded to *Pseudomonas*, *Rhodococcus*, *Actinomyces*, and *Bacillus* strains; randomly amplified polymorphic DNA (RAPD); and denaturing gradient gel electrophoresis (DGGE) analysis demonstrated a large microbial diversity. From the degradation rates, it can be predicted that such limits will be achieved by increasing further 107–117 days of the treatments. Results demonstrated to be efficient on the restoration of contaminated soil, being an alternative to treat soils contaminated with heavy hydrocarbons.

Keywords: fuel oil no. 6, biostimulation, bioaugmentation, natural attenuation, PCR-DGGE

1. Introduction

The Food and Agricultural Organization (FAO) defines soil degradation as the loss of soil productivity characteristics, either quantitatively or qualitatively. This process can be consequences of hydraulic progression or wind erosion, nutrient depletion, desertification, salinization, and pollution, among other factors [1]. Soil conservation is necessary since many important life-related processes take place in this ecosystem, including food production, biogeochemical cycles, industrial uses, and mineral extraction, besides being the substrate of our living environment. Regrettably, these human activities are the main source of soil pollution [2–5].

Exploitation and production of petroleum products, and our dependency on petroleum hydrocarbon exploitation, have brought an increase in pollution problems due to their recalcitrant nature [6–8]. The release of petroleum hydrocarbons into environment, either accidentally or as result of human-related activities, is one of the most deleterious causes of soil and water contamination, since they can cause a severe damage to the environment. This damage includes problems related to plant development and toxicity for microbial and animal species. Additionally, toxic effects could be derived from lixiviation of petroleum products into groundwater, or bioaccumulation, which can introduce the contaminant into the food web and eventually be eaten by humans [9–12].

During the last decades, several soil restoration techniques have been proposed, including extraction of contaminants by mechanical methods, by solvent extraction, or by using the capacity of soil microorganisms to degrade hydrocarbons (bioremediation) [2, 13, 14]. Physicochemical restoration techniques can be done in situ, or the contaminated soil can be removed and the treatment can be done ex situ. Among physicochemical restoration techniques, mechanical separation, electro remediation, cofferdam system, and soil cover/insulation can be included [10, 11, 15]. The objective of chemical remediation is to convert the contaminant into a substance with lower toxicity; soil washing, immobilization, chemical and photochemical reduction, and soil flushing are techniques included in this type of treatment. Both physical and chemical techniques have high associated costs and cannot remove the contaminant completely from the site [9–12].

On the other hand, bioremediation has proven to be a cost-effective, environmental-friendly, and simple technique to restore hydrocarbon-contaminated soil sites [16, 17]. Bioremediation techniques include the use of nutrients to increase microbial activity (biostimulation), the addition of an enrichment culture of contaminant-degrading microorganisms (bioaugmentation) or monitoring of the place, sometimes with air injection to promote the natural degradation process (natural attenuation). Limitations encountered in bioremediation include nutrient availability, but they can be supplied as inorganic salts [2, 5]. For hydrocarbon biodegradation in soil, the use of earthworms [16], rhizospheric microorganisms [18], the addition of surfactants and fertilizers [19], composting [20, 21], and landfarming as well as the effect of the type of soil or temperature have been evaluated [20, 22].

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In México, it has been reported that as a result of years of activity of the basic chemical industry, petrochemicals, mining, and hydrocarbon refining, an area of 25,967 km^2 of degraded soil was reported in 1999 [6]. In the northern state of Chihuahua, Mexico, an accidental release of petroleum hydrocarbons was reported in 2010 as consequence of a clandestine poaching of a hydrocarbon pipeline [8]. In one of the companies that is settled in Chihuahua City, a spill of fuel oil no. 6 from an underground storage tank, which was leaking from non-hermetic joints, was discovered during a volunteer environmental auditing process by PROFEPA (Mexican Federal Environmental Authority). Site restoration was requested, to achieve a maximum of 3.5 g/kg total petroleum hydrocarbons (TPH), in agreement with the Official Mexican Standard that refers to the levels of soil contamination allowed in industrial settings and sites that are contaminated (NOM-EM-ECOL-138-2002) [23].

Fuel oil no. 6 (FO6) is one of the last products obtained in petroleum distillation and is reported as saturate, aromatic, resin, and asphaltene (SARA) fraction, with a concentration of around 25% of saturated hydrocarbons, 35% of aromatics hydrocarbons, 20% of resins, and 20% of asphaltenes [24, 25].

The aim of this work was to evaluate at laboratory scale, and three biodegradation treatments for soil heavily contaminated with fuel oil no. 6 were done: (1) natural attenuation, (2) Biostimulation, and (3) bioaugmentation; also, the influence of the different treatments on the soil microbiota was evaluated.

2. Materials and methods

2.1. Microcosm construction

Three laboratory-scale bioreactors were constructed, to hold 6.5 kg of soil contaminated with fuel oil no. 6. An air distribution system with a flexible pipeline was built and incorporated into each microcosm. The microcosm set up as natural attenuation (control) was irrigated with 100 mL of water every 48 h. The biostimulation microcosm was irrigated every 48 h with 100 mL of a fertilizer solution containing ammonium phosphate and urea to a final concentration of 0.03% (w/v) nitrogen and 0.01% (w/v) phosphorous. In order to evaluate bioaugmentation system, a 100 mL enriched culture of soil microorganisms grown in a system with FO6 as carbon source and the same fertilizer solution were added once a week. The enriched culture was prepared adding 5 g of FO6 contaminate soil in 200 mL of fertilizer solution; FO6 saturated air was bubbled into the system (**Figure 1**).

2.2. Physicochemical soil characterization

Soil contaminated with FO6 was characterized by standard techniques, to determinate pH, texture, apparent density, nitrate, and phosphate, among other characteristics. Heavy metals were quantified by flame atomic absorption (Model Avantel Sigma, GBC, Hampshire, IL, USA); As, Hg, and Se were determined by FAAS-HG, using NIST certified standards.

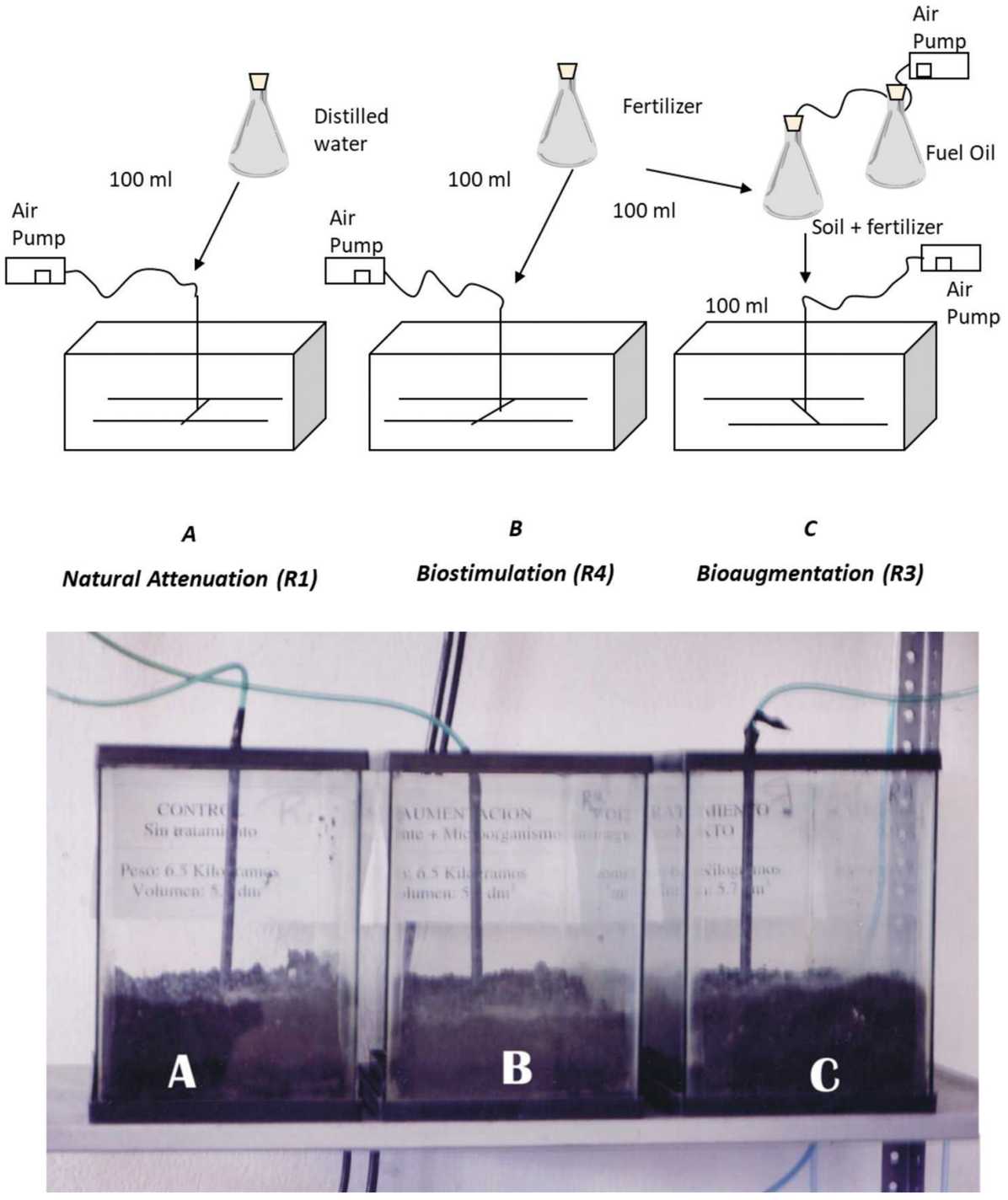

Figure 1. Lab-scale bioreactor construction. (A) Natural attenuation, (B) biostimulation, and (C) bioaugmentation.

2.3. Microbial quantification of total mesophilic bacterial count and hydrocarbon-degrading mesophilic bacteria

The quantification of total mesophilic aerobic microorganisms, as well as hydrocarbon-degrading aerobic microorganisms, was done every 15 days for 1 year. A composite soil sample (20 g) was obtained from 2 to 5 cm depth into the soil column at each bioreactor. Standard

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plate-counting method using serial dilution and spread plate technique were used. Standard Methods Agar (Bioxon, Mexico) was used for total mesophilic count, and for hydrocarbon-degrading bacteria, M9 minimal agar without carbon source added was utilized [23]. Plates were incubated in a closed chamber with a FO6-saturated atmosphere at 28°C for 48 h in the case of total mesophilic count and 7–10 days for hydrocarbon-degrading microbial counts. Results were expressed as log CFU/g of soil. From the hydrocarbon-degrading counts, representative colonies were isolated and streaked into nutrient agar (Bioxon, Mexico) to obtain isolated colonies. Macroscopic and microscopic morphology, Gram stain, as well as oxidase and catalase biochemical tests were employed for a preliminary identification of the isolates. Isolated strains were evaluated for FO6 degradation capacity using an M9 agar without carbon source added and with a top layer of FO6; bacteria were directly inoculated into the agar using a sterile toothpick, and growth was determined after 5 days of incubation at 28°C [26].

2.4. Randomly amplified polymorphic DNA (RAPD) analysis and denaturing gradient gel electrophoresis (DGGE) of hydrocarbon-degrading bacterial isolates

Isolated bacterial strains of hydrocarbon-degrading bacteria were grown in trypticase soy agar (Bioxon, Mexico), and after an incubation of 12 h at 28°C, cells were collected by centrifugation; 200 μL of lysis buffer (Tritón X-100 at 2%, SDS 1%, NaCl 10 mM, EDTA 1 mM) and 200 μL of PBS-phenol-saturated solution were added. The samples were mixed in vortex for 4 min and incubated at 65°C for 1 h. After incubation, 200 μL of TE was added, and tubes were centrifuged; the aqueous phase was transferred to a clean tube. DNA in the aqueous phase was precipitated with ethanol 70% with incubation at −20°C overnight and was later recovered by centrifugation and suspended in 400 μL of TE added with 30 μg of aRNAs. Samples were incubated at 37°C for 5 min, and the DNA was precipitated with isopropanol and recuperated by centrifugation. The pellet was air-dried and suspended in molecular biology water. This DNA was used for PCR amplification and DGGE and RAPD analysis [27]. PCR amplification for DGGE analysis was performed using primers MAR (5′ GCG CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG 3′) and MAR 1 (5′ ATT ACC GCG GCT GCT GG 3′). Amplification mixture was prepared with buffer PCR (1×), $MgCl_2$ (3 mM), dNTPs (0.2 mM), primers (0.4 pM), Taq polymerase (1 U/μL), and DNA (100 ng). Reactions were processed with an initial denaturation step (92°C for 5 min) followed by 35 cycles of denaturation (92°C for 45 s), annealing (55°C for 30 s), and elongation (72°C for 45 s) and then a final elongation step (72°C for 5 min).

DGGE analysis was done using 1-mm-thick, 16 × 16 cm polyacrylamide gels [7.0% (v/v) acrylamide-bisacrylamide (37.5/1); denaturant (urea/formamide)]. From the PCR products, 25 μL was added on denaturing gradients of 58–50%. A mixture of 7 M urea and 40% formamide was defined as 100% denaturant. The gels were run in 0.5× TAE (40 mM Tris-acetate and 1 mM EDTA) at 45 V for 20 h at 60°C. Thereafter, the gel was stained with silver nitrate (silver satin plus Bio-Rad).

For the RAPD analysis, primers F (5′-GGACTGCAGA-3′) and R (5′-AGCTGACCGT-3′) were used. Amplification mixture included buffer PCR (1×), $MgCl_2$ (3 mM), dNTPs (0.2 mM), primers (0.4 pM), Taq polymerase (1 U/μL), and DNA (100 ng). PCR was processed with 42 cycles

of denaturation (92°C for 1 min), annealing (36°C for 1.5 min), and elongation (72°C for 2.5 min) and then a final elongation step (72°C for 5 min). PCR products were analyzed in agarose gel (1.5%) in TBE (45 mM Tris-borate/1 mM EDTA). The gel was stained with ethidium bromide solution (0.5 μg ml^{-1}).

2.5. Hydrocarbons quantification

Total petroleum hydrocarbon (TPH) concentrations were determined by the 8440 EPA method gravimetric method [28], as well as by the 3570 EPA method, based in Fourier transform infrared (FT-IR) analysis [29]. For the gravimetric method, to 5 g of Na_2SO_4-dried soil sample, 25 mL of methylene chloride was added, and the mixture was shaken at 35 RPM for 1 h. Later, samples were filtered in fiberglass; the dissolvent was evaporated, and the sample was weighted. Extracted hydrocarbons obtained from the previous step were used for FT-IR quantification, by adding 4 mL of tetrachloroethylene, and evaluated in the total hydrocarbon analyzer (Model HC-404, Buck Scientific, East Norwalk, CT, USA). A standard curve was prepared using 5000–20,000 mg/kg of n-hexadecane as standard; lecture was taken at 2927.37 cm^{-1} peak. Also, a qualitative FT-IR analysis was done to determine the presence of functional groups that can describe the compounds present in FO6 (Model Spectrum GX, Perkin Elmer, Waltham, MA, USA).

2.6. Hydrocarbon degradation analysis by differential thermal analysis (DTA) and thermal gravimetric analysis (TGA)

In order to analyze the change in hydrocarbon composition, DTA and TGA analyses were done from samples obtained in the gravimetric TPH analysis. Samples were analyzed from room temperature to 700°C at a rate of 15°C/min in an air atmosphere in a TGA instrument (Model 2960, TA Instruments, New Castle, DE, USA).

3. Results and discussions

3.1. Physicochemical soil characterization

The soil profile of the FO6-contaminated soil was of an alkaline (pH 8.9) loam soil (sand 38%, silt 29%, clay 32%). Heavy metal concentrations were low (**Table 1**). Selection of remediation strategies depends largely on the extrinsic characteristic of the contaminated site, such as pH, temperature, and nutrient concentration, among other factors [3, 30], since the hydrocarbon biodegradation rate is important. The pH value depends on the organic material content and soil nature; a basic pH in soil contaminated with hydrocarbons has been found. Soil characteristics such as hydraulic conductivity, water retention, and porosity are also important for nutrient and air distribution that are essential for microbial activity. According to the results presented in **Table 1**, hydraulic conductivity is very low. It has been described that soil with high hydrocarbon concentrations presents changes in water retention and hydraulic conductivity properties [6, 31–33]. This phenomenon interferes with biological nutrient cycling, because biological remediation process depends on nutrient distribution for microbial growth.

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Physicochemical parameters	pH ($CaCl_2$ 0.01 M)	8.98
	Hydraulic conductivity (cm/h)	0.56
	Electric conductivity (mmhos/cm)	10
Inorganic ion content	$N–NO_3$ (kg/ha)	367
	P (kg/ha)	12.52
	$CaCO_3$ (%)	24.94
	K (ppm)	53.75
Textural soil classification	Clay (%)	32
	Silt (%)	29
	Sand (%)	38
		Clayey
Metal content	As (ppm)	0.01
	Ba (ppm)	3.6
	Cd (ppm)	<0.025
	Ni (ppm)	<0.1
	Hg (ppm)	<0.005
	Ag (ppm)	<0.1
	Pb (ppm)	<0.1
	Se (ppm)	<0.001

Table 1. Physicochemical characterization of soil contaminated with fuel oil no. 6.

3.2. Microbial growth in the lab-scale bioreactors and preliminary identification of hydrocarbon-degrading microorganisms

Microbial counts of total mesophilic and hydrocarbon-degrading microorganisms were evaluated in the three different lab-scale bioreactors every 15 days. The number of microorganisms was similar in the three systems, as observed in **Figure 2**, but in all treatments, an increase in microbial numbers was observed. It was also evident that a large proportion of the microbial population was able to grow using petroleum hydrocarbons as carbon source, as observed by the number of hydrocarbon-degrading bacteria (**Figure 2**). All treatments showed an increase in the microbial count up to the day 280 of treatments, and a slight decrease was observed during the rest of the study.

In order to characterize the diversity and similarity of hydrocarbon-degrading microorganisms present in the different treatments, bacterial strains with differences in morphological characteristics were isolated (Gram stain), and their distribution along the experiment is described in **Figure 3**. The higher microbial diversity was found in the first and the last trimester. The initial metabolic characterization (oxidase and catalase tests) along with morphological characterization showed the presence of *Bacillus*, *Pseudomonas*, *Rhodococcus*, and *Actinomyces* strains.

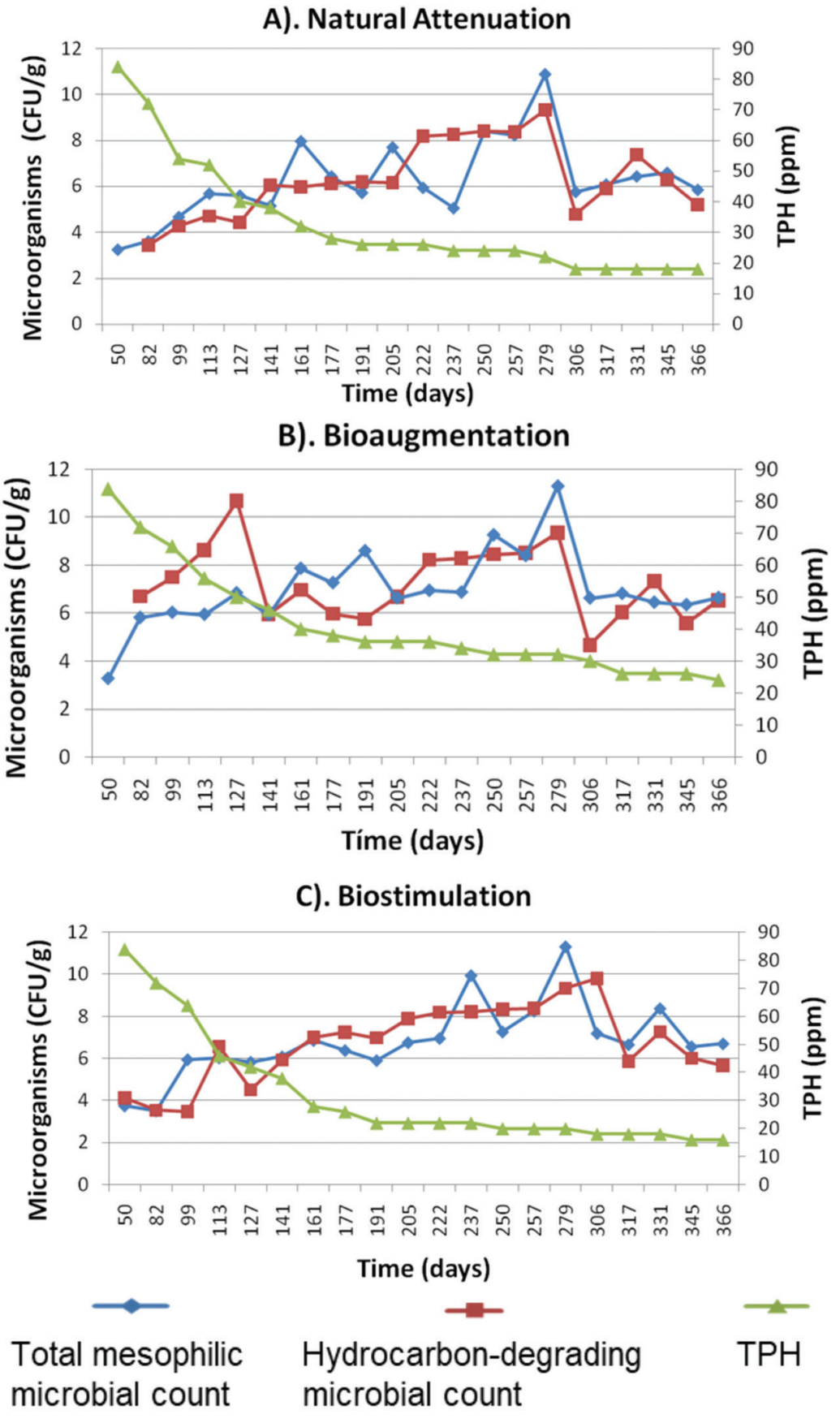

Figure 2. Microbial growth (total mesopilic and hydrocarbon-degraders) and hydrocarbon degradation (TPH) in lab-scale bioreactors. (A) Natural attenuation, (B) bioaugmentation, and (C) biostimulation.

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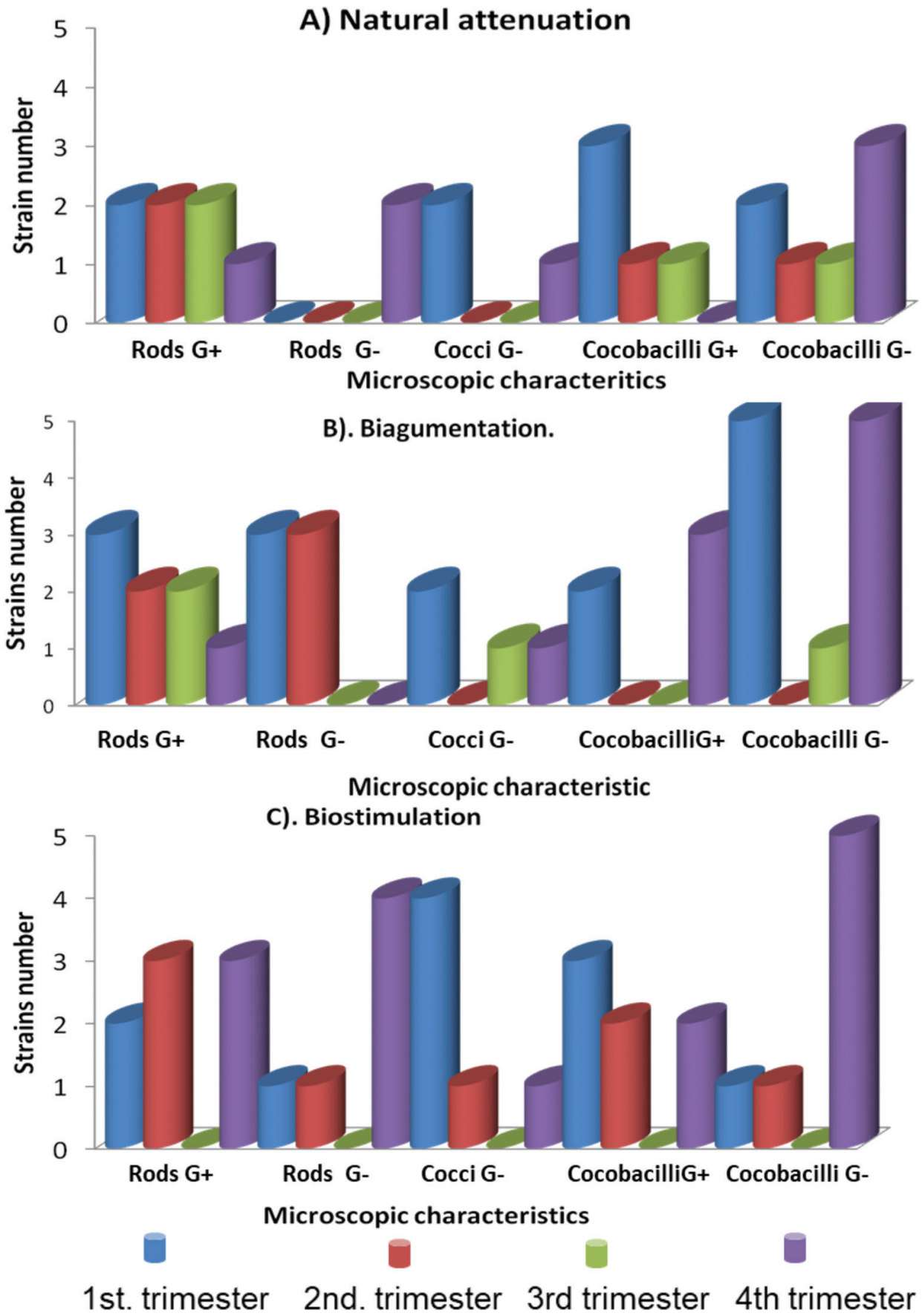

Figure 3. Preliminary characterization of microbial strains isolated from lab-scale bioreactors. (A) Natural attenuation, (B) bioaugmentation, and (C) biostimulation.

For DGGE and RAPD analysis, bacterial strains representative of the different treatments and at different sampling times were selected, to verify the diversity of microorganisms present. Results are shown in **Figure 4**, where strain identification shows the bioreactor (R1, natural attenuation; R3, bioaugmentation; R4, biostimulation), as well as the sampling period (M). As shown in **Figure 4**, the RAPD analysis and DGGE analysis present a high diversity in all the strains isolated from the different treatments. The RAPD analysis showed that strains in lines 3 and 5 have the same pattern, but the rest of the strains showed a different pattern, indicating that microbial strains are different (**Figure 4A**). However, DGGE analysis showed a high similarity as shown in the lines 5–7 and 15–18 (**Figure 4B**). The differences are due to differences on the DNA regions amplified in each technique.

It has been reported that microorganisms from the *Pseudomonas* group had a big pathway of degradation for many types of pollutants [34]. This microbial group can degrade a wide variety of hydrophilic and hydrophobic compounds, including polycyclic aromatic hydrocarbons

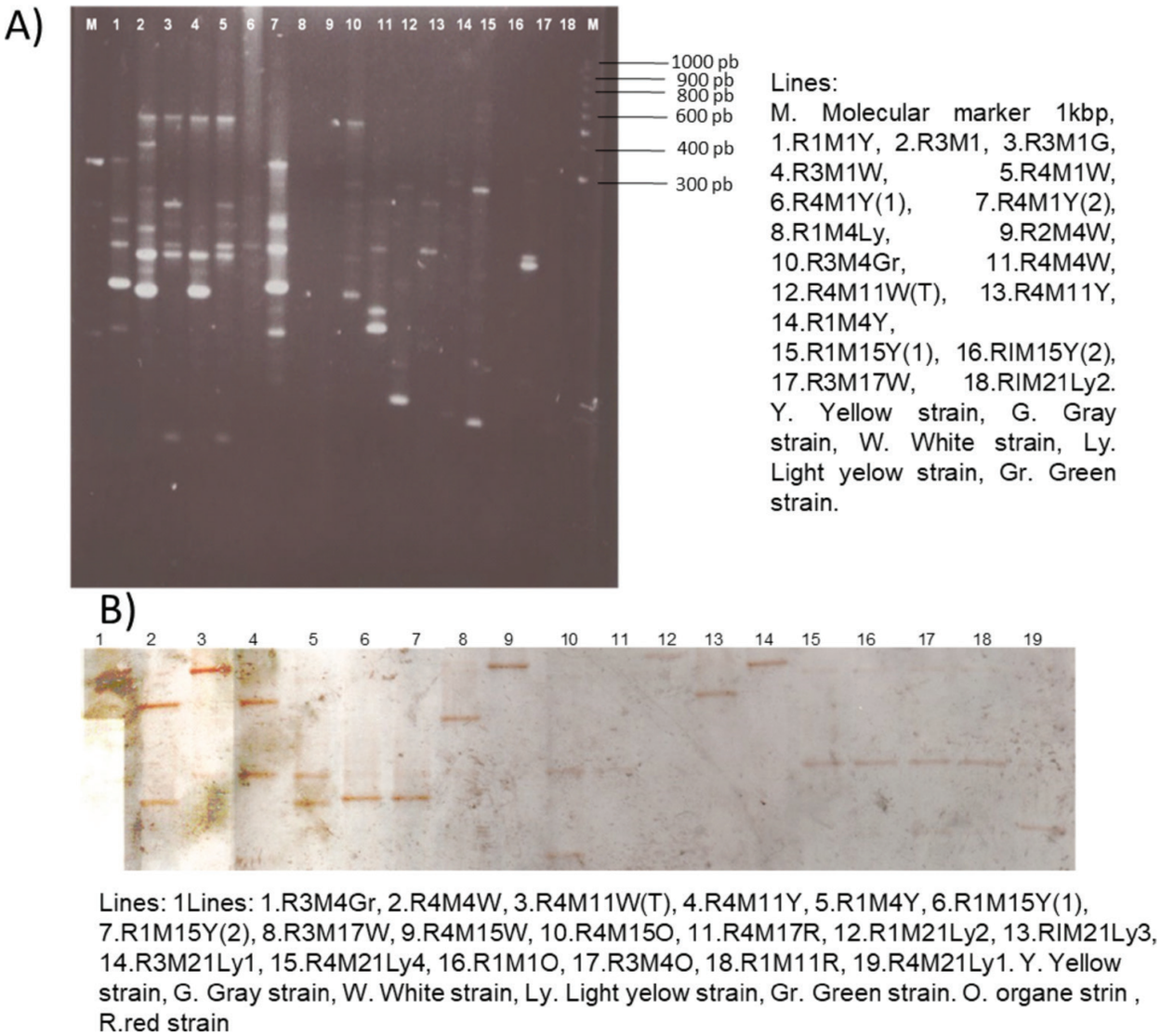

Figure 4. Analysis of hydrocarbon-degrading bacterial strains isolated from the different biodegradation treatments (A) DGGE and (B) RAPD. Strain identification is related to the bioreactor (R1, natural attenuation; R3, bioaugmentation; R4, biostimulation) as well as the sampling period (M); they are also identified by the color of the colony.

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(PAHs) [35–37]. On the other hand, *Bacillus*, *Rhodococcus*, and *Actinomyces* strains can also degrade PHAs [37–39]. The isolated strains from the soils of the three types of experiments were cultivated in M9 minimum agar added with FO6, in order to observe if they can use TPH present in FO6 as carbon source (**Figure 5**). TPH utilization was visible by a zone of degradation around the bacterial growth; in some cases, emulsification of TPH was observed, due to the production of biosurfactants by the microorganism [19, 34]. It has been reported that in hydrocarbon-degrading microorganisms, biosurfactant production allows strains to make the hydrophobic carbon source available for biodegradation [19, 40–42]. This is especially important in the degradation of complex recalcitrant compounds, such as the ones present in FO6.

3.3. Total petroleum hydrocarbon degradation

In order to evaluate the efficiency of each treatment, TPH was quantified along the time of biological treatment. Two methods were compared for TPH quantification, the gravimetric method and the FT-IR method; results showed a correlation between both methods, with a highly significant correlation coefficient ($r = 0.97$, $p < 0.01$). In the three biological treatments, TPH diminished with respect to time of treatment (**Figure 2**). The higher percentage of TPH degradation was for bioaugmentation (86.6%), followed by biostimulation (86.6%) and finally by natural attenuation (85.5%). For the three treatments, a two-rate of TPH degradation process was observed, with a higher degradation rate during the first 145 days and a slower rate during the rest of the experiment (**Table 2**). TPH values achieved in the year of study were

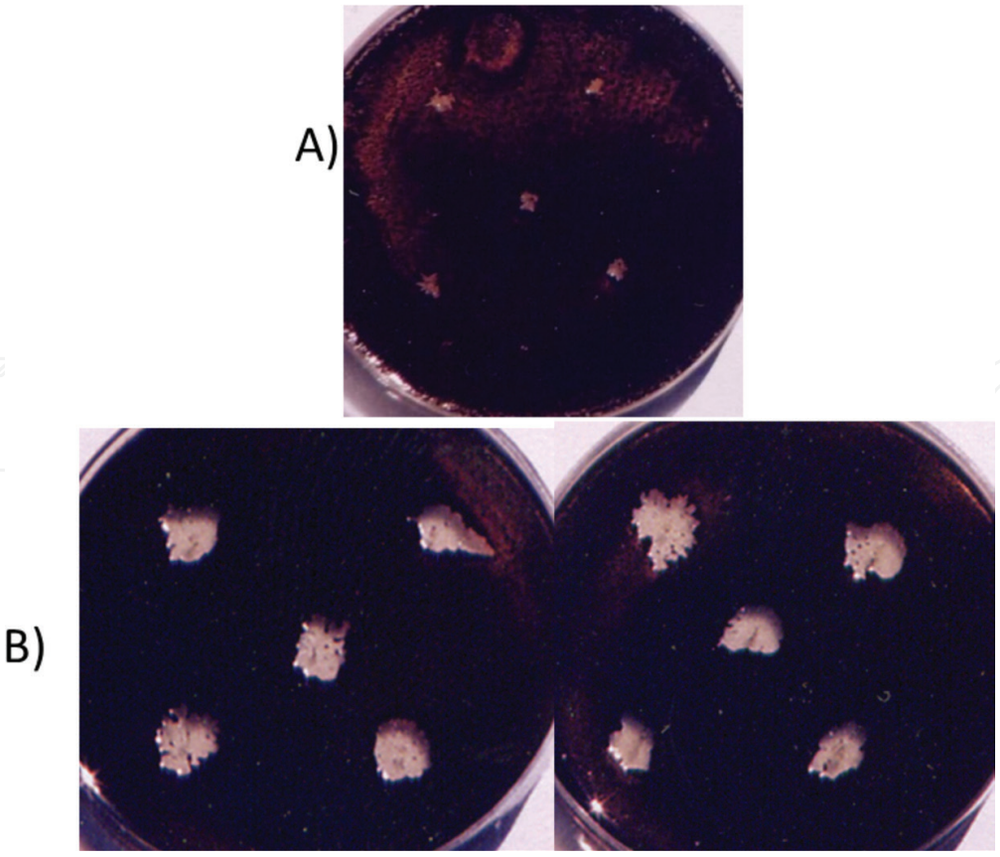

Figure 5. Analysis of hydrocarbon-degrading bacterial strains isolated from the different biodegradation treatments A) Hydrocarbon degradation but no biosurfactant production. B) Hydrocarbon degradation and biosurfactant production.

near to the values allowed by Mexican regulations [43] for soil. The rate of degradation was better in the first 145 days in all the microcosms, and in the last period of time, this value decreases (**Table 2**). These results showed that in the first 145 days of process the better treatment is bioaugmentation; nevertheless, their rate decreased in the last days.

It has been described that the use of different bioremediation strategies such as bioaugmentation, natural attenuation, or biostimulation does not produce differences in the decrease of TPH in contaminated sites [17]. On the contrary, it has been reported that bioaugmentation was the second best treatment for the removal of hydrocarbons in contaminated sites and that natural attenuation was the less effective [7]. The use of plants in combination with other biodegradation techniques, such as bioaugmentation, landfarming, assisted phytoremediation, or phytoremediation, has shown to have TPH removal efficiencies of 86, 68, 68, and 59%, respectively [7, 22, 44]. In addition, the use of compost or food waste to increase nutrient availability has also showed to be efficient in TPH removal from soil [45].

The adsorption capacity of soil, to integrate organic compounds into their particles, has been described. This function restricts the availability of contaminants to microorganisms for degradation and is, therefore, a conditional step for biodegradation: the pollutant cannot be degraded faster than it is liberated from the soil particles [46, 47]. Also, degradation can be related to microbial growth. In **Figure 2**, microbial growth of hydrocarbon-degrading bacteria decreased, and at the same time, the rate of hydrocarbons degradation was slower. The relation directly between TPH and microbial growth has been reported [48].

3.4. Hydrocarbon characterization

In order to evaluate the change in TPH composition throughout the experiment, a TGA and DTA analysis was done. The analysis of TPH found in soil at the beginning of the experiment showed a decrease in weight at 500°C and remains constant until 550°C; another weight decrease is evident until 625°C. DTA analysis showed a series of bands in the range of 450–500°C (**Figure 6A**). These results indicated that TPH was composed of hydrocarbons with aliphatic chains; this result is similar with the description done in the Prestige oil spill, where the presence of PAH was described in FO6 [25].

	Rate of biodegradation of HTP (mg of HTP/day)			**Time (days) required for closure***
	0–145 days of experimentation	**145–366 days of experimentation**	**0–366 days of experimentation**	
Natural attenuation	207.6	50.108	112.5	96
Bioaugmentation	225.3	42.5	114.9	93
Biostimulation	208.6	51.21	113.5	86

*Time (days) required to reach the value of HTP specified by Mexican normativity [43].

Table 2. TPH degradation in a soil contaminated with fuel oil no. 6, subjected to three bioremediation treatments at lab-scale bioreactors.

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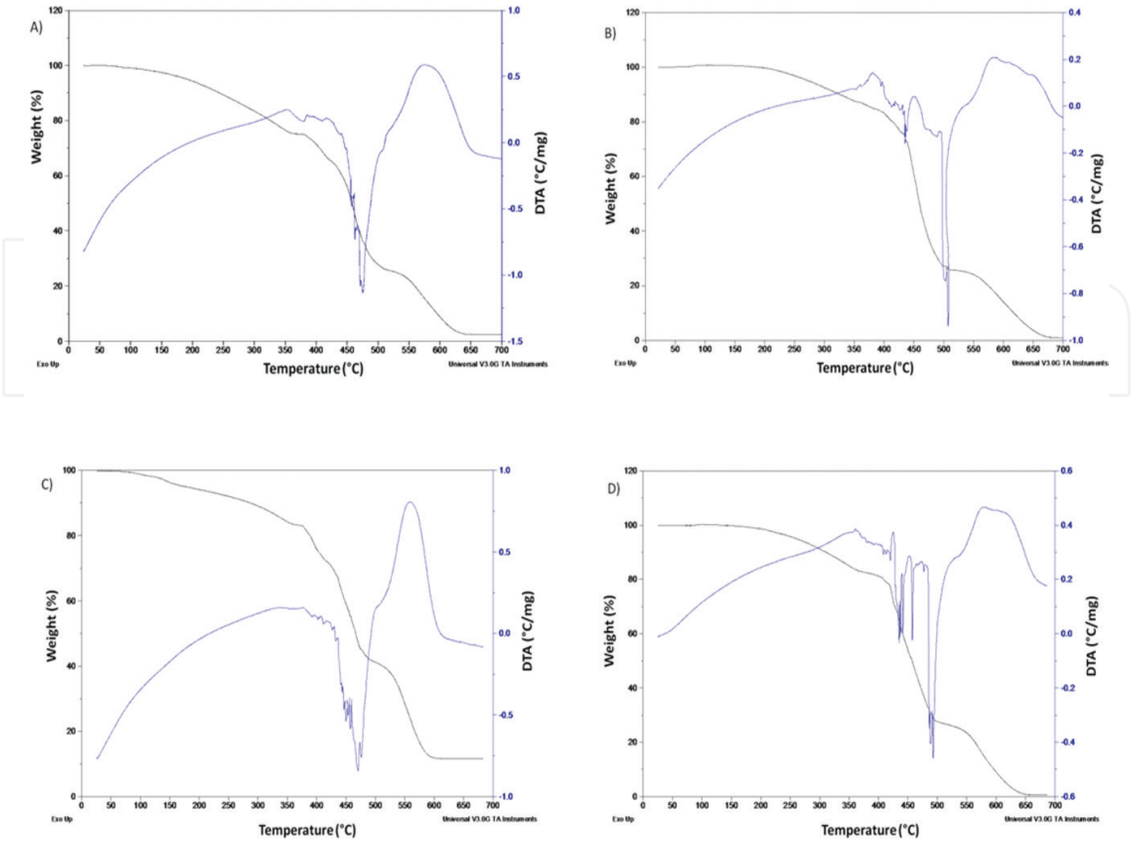

Figure 6. Analysis of TPH by DTA and TGA in the different treatments. (A) Initial TPH analysis. TPH remaining after 366 days of biological treatment, (B) natural attenuation, (C) bioaugmentation, and (D) biostimulation.

The results of the DTA and TGA analysis of the remaining TPH after 366 days of biological treatment indicated differences among bioremediation techniques used. Natural attenuation showed bands in the range of 425–475°C, with a bigger number of signals than at the beginning of the experiment (**Figure 6B**). In the case of bioaugmentation and biostimulation (**Figure 6C** and **D**, respectively), results were similar. The increase in the number of bands and the shift in degradation temperatures are the result of transformation of large hydrocarbon molecules into others of lower molecular weight. On the other hand, once the shorter molecules are consumed in the contaminated site, the use of more complex molecules is initiated; this is reflected in the TGA and DTA analysis by the decrease in evaporation temperature in the bands.

On the other hand, FT-IR analysis not only showed TPH decrease in concentration, but was also used to establish a relationship between hydrocarbons with CH_3—CH_2 groups and the presence of CH_3 residues. The band at 1459.52 cm^{-1} is the representative of the CH_3—CH_2 link, and the band in 1377.03 cm^{-1} is the representative of CH_3 residue. The relation between the CH_3 and CH_2/CH_3 can be used to express the conversion of compounds with differences in the proportion of those functional groups. Results are shown in **Table 3**, where an increase of CH_3 is observed in all treatments.

Treatment	Time of treatment (days)	CH_2—CH_3/CH_3 ratio
Natural attenuation	0	1.70
	93	1.19
	184	1.12
	275	1.13
	366	1.14
Bioaugmentation	0	1.70
	93	1.18
	275	1.10
	366	1.15
Biostimulation	0	1.70
	93	1.19
	184	1.14
	366	1.20

Table 3. FT-IR analysis of TPH from the three lab-scale bioreactors for bioremediation strategies, in soil contaminated with FO6. The relationship in band height (1459.52 cm^{-1}/1377.03 cm^{-1}) is shown as CH_2—CH_3/CH_3 ratio.

Relationship of functional groups has been used for the determination of the relation between C and H [42]. The change in bands in the TGA and DTA analyses, as well as the increase in CH_3 observed by FT-IR, suggests that the transformation of TPH was mediated by the soil microbiota. Bioremediation strategies such as the ones used in this study have been successfully used in site restoration [48–50].

4. Conclusion

Soil is a nonrenewable resource; it is therefore important to generate restoration strategies in sites that have been contaminated due to human activities, where soil quality and functionality need to be restored. Bioremediation of soil contaminated with hydrocarbons can be a time-consuming technique but is cost effective and environmentally appropriate, since the contaminants are transformed by soil microorganisms into simple noncontaminant organic compounds. In this work the degradation of TPH was evaluated by three treatments: natural attenuation, biostimulation, and bioaugmentation. After 1 year of study, degradation was higher for biostimulation (0.19 g TPH/day), followed by natural attenuation (0.18 g TPH/day) and bioaugmentation (0.16 g TPH/day). *Pseudomonas*, *Rhodococcus*, *Actinomyces*, and *Bacillus* were present in the reactors, according to preliminary identification of bacteria, with a large microbial diversity, as observed by molecular culture-independent techniques. Based on qualitative analysis of TPH, a change in the length of the aliphatic chain was observed, as a result of microbial activity. It is always important to establish the best bioremediation method to be used in each particular site, since the success in a bioremediation process depends on environmental factors as well as on the composition of the microbiota present *in situ*.

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