Bull Environ Contam Toxicol (2017) 98:698–705
DOI 10.1007/s00128-017-2039-0

Biodegradation of Diesel, Crude Oil and Spent Lubricating Oil by Soil Isolates of *Bacillus* spp.

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Received: 5 October 2016 / Accepted: 30 January 2017 / Published online: 16 February 2017

Abstract Two species of *Bacillus, B. thuringiensis* B3 and *B. cereus* B6, isolated from crude oil-contaminated sites in Ecuador, were tested for their capability in degrading polycyclic aromatic hydrocarbons (PAHs) in diesel (shake-flask), and to remove total petroleum hydrocarbons (TPHs) from crude oil- or spent lubricating oil-polluted soils (plot-scale). TPHs and PAHs were analyzed by Gas chromatography-Flame ionization detector (GC-FID) and High performance liquid chromatography (HPLC), respectively. Degradation percentages of PAHs by strain B6 were in the range of 11–83 after 30 days. A mixed culture of both the strains removed 84% and 28% of TPHs from crude oil- and spent lubricating oil-polluted soils, respectively. Reduction in the abundance of total *n*-alkane fractions (C_8–C_{40}) of spent lubricating oil was 94%, which was 18% higher than the control. Our results clearly indicate that the selected strains have great potential in degrading petroleum hydrocarbons at both laboratory- and field-scales.

Keywords *Bacillus* spp. · Biodegradation · Plot-scale study · Petroleum hydrocarbons

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Pollution by petroleum hydrocarbons (PHs) in Amazon rainforest soils of Ecuador was a major concern for many years (Gesinde et al. 2008). This country is the home for the third largest oil reserves in South America (Lloyd 2015), and ranked fifth in South and Central America (CIA 2015). Crude oil operations (petroleum extraction and transportation) in Ecuador resulted in discharging about 30 billion gallons of industrial waste products onto soil and into the surrounding water bodies (Sebastian and Hurtig 2004). In addition, Ecuador has plans for drilling operations in one of its most biologically diverse areas, Yasuni National Park (Larrea and Warnars 2009) which is the home for many groups of indigenous people such as Huaorani. Therefore, ecosystems here with rich biodiversity are under threat from oil exploitation. Unfortunately, research on bioremediation of crude oil-contaminated soil is scarce in this country. Although very few studies reported on microorganisms present in Ecuadorian oil-fields (Buccina et al. 2013), the interactions between indigenous Amazonian microflora and PHs have not been investigated.

Field-level bioremediation experiments cannot be conducted without establishing the potential of the microorganisms. In contaminated areas where PHs-degrading microorganisms exist, bioremediation can be augmented by introducing exogenous microorganisms through the approach of bioagumentation (Supaphol et al. 2006). At present, there is considerable interest on identification of microorganisms that degrade PHs, particularly, the recalcitrant fractions of crude oil and lubricants, because they are the critical components in influencing the success rate of bioremediation. Especially, gram-positive bacteria such as *Bacillus* spp. may be used as candidates for biodegradation of more recalcitrant PAHs that occur predominantly in petroleum products (Kostka et al. 2011). For example, *B. cereus* ACE4 degraded almost all the *n*-alkanes (C_{10}–C_{20})

and many of the branched alkanes found in diesel (1%) in 30 days (Rajasekar et al. 2007). In another study, *Bacillus cereus* DRDU1 degraded 71% of diesel and 64% of crude oil in 4 weeks (Borah and Yadav 2014). While investigating the efficiency of *B. thuringiensis* for degrading phenanthrene, Laura et al. (2016) found 80% removal of this model PAH in batch culture. Degradation studies focusing on complex mixtures of PHs will be more useful than those involving individual components. Therefore, two strains of *Bacillus* spp., *B. thuringiensis* B3 and *B. cereus* B6, previously isolated from Ecuadorian oil-fields (Maddela et al. 2015), were assessed for their potential in degrading diesel, crude oil and spent lubricating oil. In particular, biodegradation of PAHs from diesel in shake-flask cultures, and TPHs from crude oil-(artificial contamination) and spent lubricating oil-contaminated soil (natural contamination) using mixed cultures of these two strains in plot-scale, was investigated in this study.

Materials and Methods

Two strains of bacteria, *B. thuringiensis* B3 and *B. cereus* B6, used in the present laboratory and plot-scale (under field conditions) studies were maintained on nutrient agar medium. For the a plot-scale study, soil (~100 kg) with no known history of any oil contamination was collected from a local area (Centro de Investigación, Posgrado y Conservación Amazónica (CIPCA), Canton Santa Clara/Arosemena Tola, Province Pastaza/Napo, Ecuador). The physico-chemical characteristics of the soil were: pH, 6.4; sand, 69.0%, silt, 18.0%; clay, 13.0%; moisture content, 56%; nitrogen, 0.75%; phosphorous, 48 μg g^{-1}; potassium, 457 μg g^{-1}, and organic carbon, 2.97%. For another second plot-scale study, spent lubricating oil-contaminated soil (~100 kg) was collected at an automobile workshop in the city of Ambato, Tungurahua, Ecuador. The physico-chemical characteristics of this soil were: pH, 4.0; moisture content, 45%; density, 0.5 g cm^{-3}; electrical conductivity, 0.1 Sm^{-1}; $N0_3^-$-nitrogen, 20 mg L^{-1}; NH_3^+-nitrogen, 10 mg L^{-1}; total nitrogen, 30 mg L^{-1}; phosphorous, 13 μg g^{-1}; potassium, 30 μg g^{-1}; calcium, 20 μg g^{-1}; aluminium, 1.2 μg g^{-1}; and trace amounts of sulphur.

The kinetic curves were obtained by growing the bacterial strains in 100 mL (pH 7.4) portions of M9 minimal salts medium (MSM) (*SIGMA M6030*) supplemented with 1% (v/v) filter-sterilized diesel as sole source of carbon. Bacterial suspension (1 mL of 1.0 OD_{600}) obtained from logarithmically-growing cultures was used to inoculate the medium contained in 500 mL Erlenmeyer flasks. Triplicate flasks with inoculated and uninoculated media were kept in an incubator-shaker (120 rpm) at 37°C for 1 week. For every 24 h, triplicate samples (1 mL each) were withdrawn carefully from each flask without touching the diesel layer. The absorption was read at 600 nm in a spectrophotometer (Thermo Scientific™ GENESYS™ 10S UV–Vis), and growth curve was developed.

For another growth study, 10 mL of MSM with varying pH values of 2, 4, 6, 7.5, 8 and 10 were taken in separate test tubes (150×24 mm), and sterilized at 121°C for 15 min. A separate set of tubes, in triplicate, was used for each bacterial strain. Then, 200 μL of diesel was mixed with the medium to provide a final concentration of 1% (v/v). Bacterial suspensions (200 μL of 1.0 OD_{600}) of the strains B3 and B6 were used to inoculate the medium in both the sets. All tubes were kept at 37°C for 4 days, and the absorption was read at 600 nm in a spectrophotometer. Similarly, growth of both the bacterial strains was determined under the influence of different temperatures (8, 25, 37 and 55°C), concentrations of diesel (0.5%, 1.0%, 1.5%, 2.0% and 2.5%, v/v) and nutrients of NPK (Yaramila™ complex 12-11-18 at 2, 4, and 6 g L^{-1}, w/v). In all these experiments, the culture medium was supplemented with 1% diesel, unless otherwise mentioned. After incubating for 4 days at 37°C, bacterial growth was determined by measuring absorbance of the culture medium. The effect of these three growth conditions was also studied by determining the colony forming units (cfu) from cultures grown in MSM supplemented with 1% crude oil.

Degradation of PAHs in diesel-supplemented medium by a bacterial strain was studied in shake-flasks. Portions (100 mL) of MSM contained in 250 mL Erlenmeyer flasks were supplemented with 1% (v/v) diesel, and inoculated with strain B3 or B6 (1 mL of 1.0 OD_{600}). Uninoculated media served as controls. All the flasks, in triplicates, were kept in an incubator-shaker at 120 rpm and 37°C. After incubation for 30 days, samples were withdrawn for quantification of PAHs by HPLC.

A plot-scale experiment was conducted to determine the potential of both the bacterial strains, in a mixture, in removing TPHs from crude oil-polluted soil. Briefly, 10 kg soil was layered (10-cm depth with 50% WHC) in a wooden box (30×45×10 cm), and thoroughly mixed with 500 mL of Tween-80 (1.0 g L^{-1} w/v) and crude oil (10% w/w, API gravity 21.4, Petroamazonas EP). In all, three sets of treatments (T1–T3) in triplicates were maintained. Treatment sets T1 and T2 served as uninoculated controls representing 0 and 90 days of incubation, respectively. After leaving the set T3 for 72 h at ambient temperature and 75%–85% humidity, 100 mL of bacterial suspension (1.0 OD_{600}) in mixture was added to soil, and thoroughly mixed to a final cell density of 10^8 cells g^{-1} soil. Finally, treatments of T2 and T3 were left in an open field (CIPCA, 17°89′38″S, 986°28′31″E, 582 m altitude) for 90 days with regular tilling using a spatula (34×9×3 cm) at 5 days intervals. Once a week, MSM was sprayed for maintaining

moisture (50%) and nutrients. Also, 250 mL of glucose solution (250 mg L^{-1}) was added to all the soil samples at 10 days intervals in order to provide a co-carbon source and to enhance the rate of PAHs degradation by microorganisms during bioremediation (Das and Mukherjee 2007). Soil samples were withdrawn from each box after 90 days of incubation, and were used for solvent extraction (Mukherjee and Bordoloi 2012) and analysis of TPHs (Joo et al. 2008).

In another plot-scale experiment, the potential of both the bacterial strains in a mixture was tested for the removal of TPHs from soil contaminated naturally with spent lubricating oil. Experimental setup and methods were similar to those described above for crude oil treatments. But, this experiment was carried out at another experimental site (9°860′366″N, 766′341″E, 2500 m altitude) close to an automobile workshop. Treatment sets used in this experiment were labeled as T4, T5 and T6 that represent 0 day uninoculated control set, 90 days uninoculated control set and 90 days inoculated set that received mixed cultures, respectively.

To determine PAHs in the culture medium withdrawn, 5 mL samples were extracted following the US EPA Method 3550B that was developed for extraction of non-volatile and semi-volatile organic compounds from medium (US 1996), and were analyzed by HPLC. Degradation potential was expressed in terms of a removal ratio (Joo et al. 2008) which was calculated as follows: Removal ratio = (Initial concentration − Residual concentration)/(Initial concentration) × 100%.

To determine TPHs in soil samples by GC, a wet sample (10 g dry equivalent weight) was mixed with anhydrous sodium sulfate (5 g) in an Erlenmeyer flask. After adding 100 mL analytical grade hexane, the flask was ultrasonically agitated. An aliquot of the hexane extract was taken into a vial and analyzed by GC-FID (Thermo Scientific™ TRACE 1300) on a HP-5 column. The injection and detector temperatures were 250 and 280°C, respectively. The oven temperature was programmed from 40 to 280°C at 12°C min^{-1}, held for 20 min, and finally maintained at 280°C for 8 min. The carrier and make up gases were nitrogen and helium, respectively, and the gas ratio was 1:10 (purge:total). TPHs were quantified by comparing the total chromatogram peak area of the sample with that of *n*-alkane standard mixture (C_{10}–C_{40}), and expressed in terms of removal ratios (Joo et al. 2008).

GraphPad Prism-7 was used for statistical analyses. Analysis of variance (ANOVA) was performed to identify the significant effects of different factors on bacterial growth, and efficiency of the strains B3 and B6 in degrading PAHs from diesel, and TPHs from crude oil and spent lubricating oil. When significant difference was indicated by ANOVA, a two-way ANOVA was used to determine whether the means of two or more groups differ. Tukey pair-wise comparisons were made using the Tukey method at 95% confidence.

Results and Discussion

Diesel was chosen for bacterial growth studies because it has been generally used as a carbon source for isolating alkane-degrading bacteria following selective enrichment (Kang et al. 2009). Since higher amounts of long chain alkanes are present in crude oil, alkane-degrading bacteria have great potential in bioremediation of crude oil-contaminated sites (Kang et al. 2009; Yoon-Suk et al. 2009). The pattern of growth, measured in terms of culture turbidity, for both the bacterial strains in M9 medium supplemented with diesel was very similar (Fig. 1a). The lag phase in growth lasted for 12 h, and the log phase continued up to 6 days resulting in an increase of growth by 4.2 times, clearly indicating that the two strains could utilize diesel as sole carbon source. Similarly, Ogochukwu et al. (2013) observed a rapid exponential growth in microorganisms isolated from diesel oil-polluted soil at a rate ranging from 0.015 to 0.094 h^{-1}.

The culture turbidity in strain B3 increased significantly after 96 h with a rise in pH from 7.5 to 8.0 (Fig. 1b). On the other hand, growth of strain B6 increased significantly ($p < 0.0001$) at pH 6. Thus, the optimal pH values for growth of both the strains on diesel were 8 and 6, respectively. Nevertheless, utilization of diesel by strain B6 was significantly more (41%) when compared with strain B3. Generally, pH extremes in soil would have a negative influence on microbial degradation of hydrocarbons. For example, the rate of biodegradation of gasoline was doubled in acidic soil (pH 4.5) after adjusting its pH to 7.4; however, the degradation rates declined significantly with a further rise in pH to 8.5 (Verstraete et al. 1975).

The strain B3 grew rapidly at 25°C in presence of 1% diesel (Fig. 1c). Maximal culture turbidities (OD_{600}) recorded after 96 h for strain B3 and B6 were 0.41 and 0.091, respectively. Thus, the growth of strain B3 was 4.5 times higher than that of strain B6 at 25°C. Temperature plays an important role on microbial degradation of PHs in contaminated sites by two ways. Firstly, it affects directly the chemistry of pollutants, and secondly, it alters the physiology and diversity of microorganisms concerned (Venosa and Zhu 2003). In general, the rate of biodegradation declines with decreasing temperatures. Highest microbial degradation occurs within the temperature range of 30–40°C in soil environments. With varying diesel concentrations, both the strains showed maximal growth, in terms of culture turbidity, at a concentration of 2% (Fig. 1d). In fact, there was a steep increase (200%) in the growth of

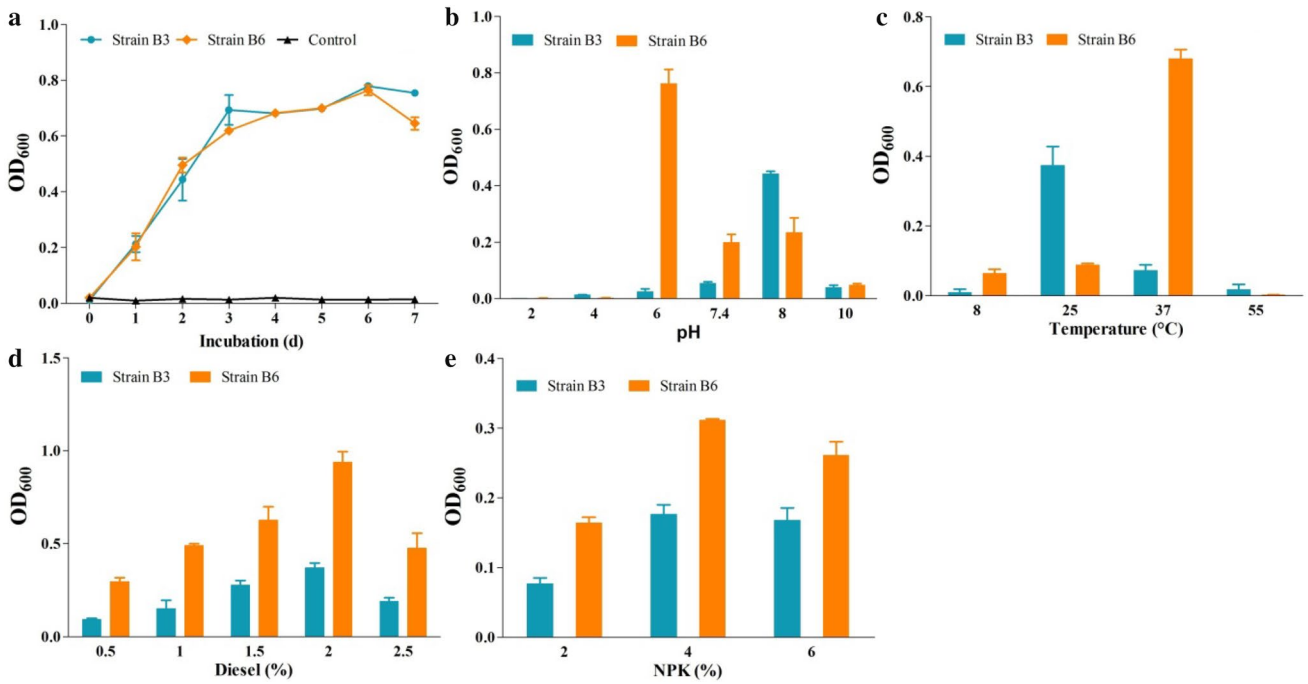

Fig. 1 **a** Growth of bacteria in 1% diesel-supplemented M9 medium. Effect of **b** pH, **c** temperature, **d** diesel concentration, and **e** NPK on bacterial growth. Error bars represent standard deviations (n = 3)

strain B6 as the concentration of diesel increased from 0.5% to 2%, but there was a significant decrease in turbidity at higher concentration of 2.5% diesel used. Similar trend was observed with strain B3 as well. These results clearly suggest that microbial degradation of diesel is greatly affected by its concentration. Rahman et al. (2002) also reported a decrease in crude oil degradation by mixed bacterial consortium from 78% to 52% with an increase in oil concentration from 1% to 10%.

In MSM supplemented with 4% NPK together with 1% diesel, both the bacterial strains grew rapidly (Fig. 1e). However, when compared with 4% NPK, higher concentration of 6% had no further positive effect on growth of strain B3 while there was a decline (28%) in culture turbidity of strain B6. Borah and Yadav (2014) reported that the cfu in *B. cereus* strain DRDU1 increased from 7.5×10^{9} to 4×10^{10} mL^{-1} upon the addition of N and P to 2% kerosene-supplemented medium. However, it cannot be ignored that the excessive concentrations of nutrients would also inhibit the bacterial degradation of hydrocarbons in petroleum (Walworth et al. 2007). In crude oil containing MSM, the optimal growth conditions, measured in terms of cfu mL^{-1}, for strain B3 were: 6 pH, 25°C temperature, 2% crude oil concentration, and 4% NPK, and the corresponding values of these growth conditions for strain B6 were: 6, 37°C, 2% and 4%. Similarly, Sathishkumar et al. (2008) reported highest per cent degradation of crude oil by *Bacillus* sp. IOS1-7 at pH 7.0, temperature 35°C and 1% crude oil. Our results indicate that the two selected strains have great potential in utilizing hydrocarbons in diesel as their sole carbon and energy sources.

The most abundant peaks appeared from uninoculated control samples (Fig. 2a) corresponded to the notable PAHs of diesel such as naphthalene (N), fluorene (F), phenanthrene (Ph), anthracene (A), pyrene (Py), benzoanthracene (BA), crisene (C), benzo-flouranthene (BF), and benzo-pyrelene (BP). However, a comparison of chromatograms obtained from samples of uninoculated control (Fig. 2a) and inoculated with strain B3 (Fig. 2b) reveal a 21% decrease in total abundance of PAHs, indicating the bacterial utilization of different PAHs present in diesel.

The data in Fig. 2c represent the extent of biodegradation of individual PAHs contained in diesel by strain B6. The per cent degradation of individual PAHs by strain B3 varied significantly. Thus, the biodegradation percentages for N, F, Ph, A, Py, BA, C, BF and BP were 19, 24, 26, 12, 11, 12, 19, 83 and 74, respectively. In spite of their complex structures and high molecular weights, both BP (6-membered ring compound, MW 276) and BF (5-membered ring compound, MW 252) were significantly utilized by the strain B6 probably due to the lowest abundance of these PAHs in diesel. Degradation of PAHs of high MW by microorganisms is not uncommon (Laila and Nour 2008). On the contrary, because of higher relative abundance of naphthalene (2-membered ring compound, MW 128) in diesel, its per cent bacterial degradation was only 19. Our

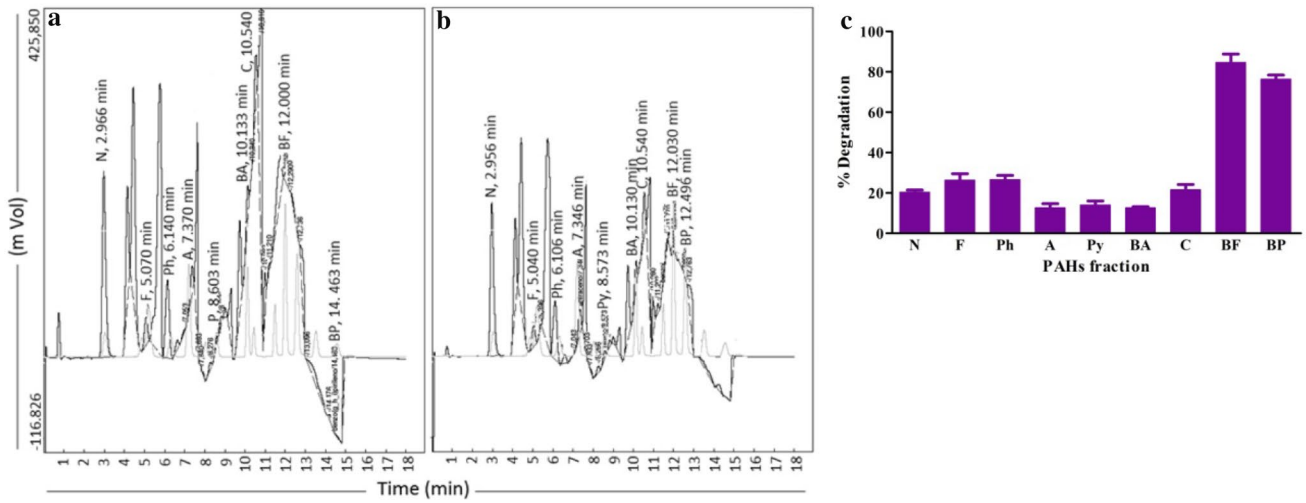

Fig. 2 Degradation of PAHs in diesel-supplemented medium by strain B6. Chromatograms of residual PAHs of **a** control and **b** strain B6, and **c** % degradation of different PAHs fractions. Naphthalene (N), fluorine (F), phenanthrene (PH), anthracene (A), pyrene (P), benzoantharcene (BA), chrysene (C), benzofluoranthene (BF), and benzopyrene (BP)

results suggest that the abundance, but not the mass and structure, plays a critical role in biodegradation of PAHs. Ogochukwu et al. (2013) reported that microorganisms isolated from diesel-contaminated soil grew poorly on medium containing nitrogen or phosphorus, but they grew well on crude oil, diesel, kerosene, engine oil, cyclohexane, and dodecanol. In another study involving pure cultures, Gomaa (2013) observed 65%–95% removal of naphthalene, phenanthrene, and pyrene by *Bacillus* sp. strain DV2-37 and *B. licheniformis* strain ABRII6 in BH medium.

Maximum and minimum air temperatures recorded during the first plot-scale experiment were 30, and 13°C, respectively. Total precipitation was 474.1 mm in 90 days. Removal of TPHs from diesel-supplemented culture medium in control samples was evident during 30 days incubation (Fig. 3a). But, inoculation of either strain B3 or B6 into the medium resulted in nearly complete removal of TPHs. Similarly, inoculation with the mixed culture (T3) was significantly ($p < 0.001$) effective in removing TPHs from the crude oil-polluted soil when compared with 0 day (T1) and 90 days (T2) controls (Fig. 3b). At the beginning of the experiment, there was $12{,}500 \pm 500$ mg TPHs kg^{-1} soil with 83% recovery of TPHs by the method employed, and this concentration declined to 1931 ± 113.5 mg kg^{-1} by the mixed culture (Fig. 3b). On the other hand, there was only 60% removal of TPHs in T2 (90 days control), indicating that the indigenous microorganisms were also effective in removing TPHs. In a lab-scale soil microcosm study, Agarry and Ogunleye (2012) observed 75.85%–84.76% removal of TPHs from crude oil-polluted (10%) soil in 6 weeks, whereas only 44.78% TPHs were removed from uninoculated controls. Additions of nutrients or biomass generally increase the rate of removal of pollutants from the contaminated sites. It was observed in earlier lab-scale

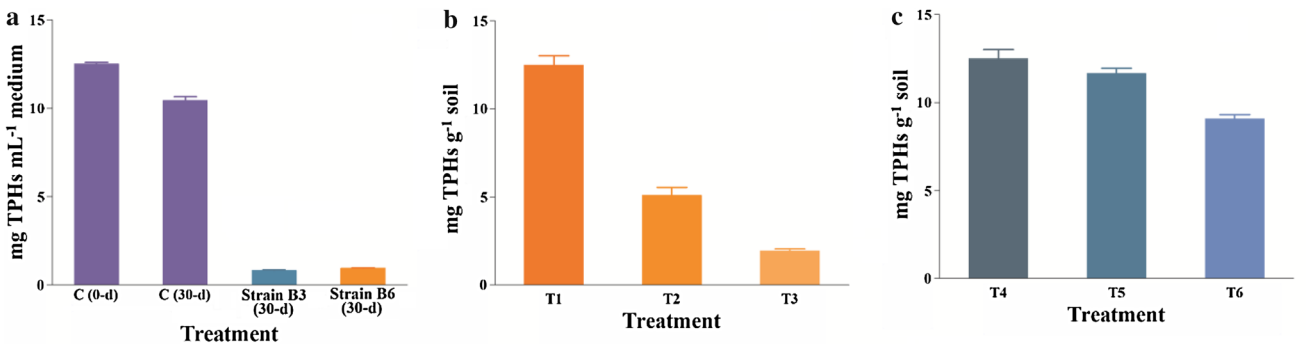

Fig. 3 Removal of TPHs by the selected bacterial strains. **a** Diesel-supplemented medium, **b** crude oil-contaminated soil, and **c** spent lubricating oil-contaminated soil. Error bars represent standard deviation (n = 3)

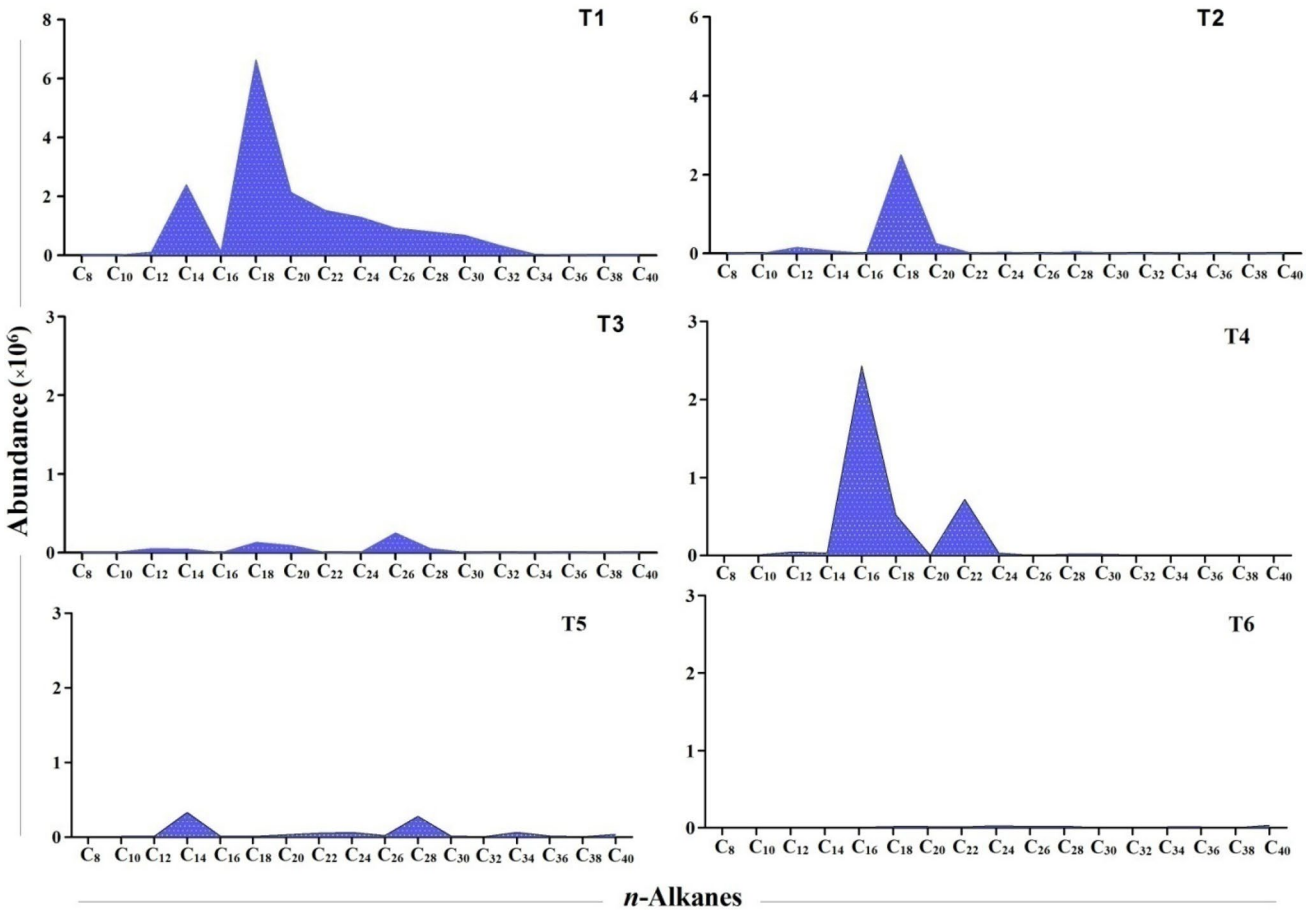

Fig. 4 Removal of *n*-alkanes from crude oil-contaminated (*T1–T3*), and spent lubricating oil-contaminated (*T4–T6*) soils in plot-scale study

studies that the present strains (B3 and B6) together with two soil fungi (*Geomyces pannorum* and *Geomyces* sp.) removed 79.47% and 87.77% of TPHs in solid- and slurry-phase, respectively (Maddela et al. 2016).

The data presented in Fig. 3c exhibit the potential of strains B3 and B6 in removing TPHs from soil polluted with spent lubricating oil in a 90 days plot-scale experiment. The maximum and minimum air temperatures (°C) and total precipitation (mm) during this study period were 22.0, 4.0 and 22.1, respectively. Unlike the first field experiment, the initial TPHs were $>12{,}500 \pm 500$ mg kg^{-1} soil (T4). The mixed inoculum of strain B3 and B6 (T6) degraded TPHs decreasing these concentrations to 9044 ± 250 mg kg^{-1}. Thus, 28% of TPHs were removed from the spent lubricating oil-polluted soil by the mixed culture in 90 days. In contrast, only 7% removal of TPHs was observed in control soil that did not receive the bacterial inoculums (T5). Lower removal rates for TPHs observed with the mixed culture from soil naturally contaminated with spent lubricating oil might be due to higher concentrations of *n*-alkanes and/or the presence of more recalcitrant hydrocarbons. Typically, lubricants contain 90% base oil (most often petroleum fractions, called mineral oils) and <10% additives (Koma et al. 2003). Base oil is rich in long chain (C_{16}–C_{36}) saturated hydrocarbons and >75% cyclic alkanes which are known to be recalcitrant to microbial degradation (Koma et al. 2003). In many occasions, toxic metals and PAHs detected in used oil inhibited the microbial degradation of hydrocarbons (Adesodun and Mbagwu 2008). Nevertheless, bacteria belonging to the genera *Alcaligenes, Acinetobacter, Arthrobacter, Bacillus, Citrobacter, Micrococcus, Pseudomonas, Rhodococcus, Serratia*, etc., isolated from hydrocarbon-contaminated sites, degraded lubricating oil (Ron and Rosenberg 2014). Recently, Bhattacharya et al. (2015) observed 48.5% degradation of waste engine oil within 7 days in shake-flasks by a bacterium, *Ochrobactrum* sp. C1, isolated from steel plant effluents.

The GC fingerprints obtained from crude oil-contaminated soil samples revealed that *n*-alkanes ranging from C_8–C_{26} were removed rapidly from T3 samples while only C_{14}–C_{16} compounds were degraded from T1 and T2 (Fig. 4). These results indicate that indigenous microorganisms contained in soil contaminated with crude oil

selectively degraded short chain *n*-alkanes. Similarly, Binazadeh et al. (2009) observed that *Rhodococcus* sp. Moj-3449 preferentially degraded short chain *n*-alkanes (C_{14}–C_{19}) from crude oil. Interestingly, there were significant changes in the abundances of *n*-alkanes in solvent extracts collected from soil samples contaminated with spent lubricant oil (Fig. 4). In fact, *n*-alkanes of C_{16}, C_{18} and C_{22} were high in their abundance in soil at the beginning of the experiment (T4). These hydrocarbons disappeared completely during 90-d incubation of soil samples inoculated without (T5) or with the mixed culture of both the strains (T6). Thus, the removal of TPHs was significant in soil samples that received the mixed culture although the range of *n*-alkanes was C_8–C_{40} in spent lubricating oil.

In conclusion, both bacterial strains reached a maximum cell density (0.6–0.7 OD_{600}) within 72 h in 1% diesel-amended MSM. Significant degradation (11%–83%) of aromatic fractions of diesel was observed with strain B6 in a 30 days shake-flask culture study. Plot-scale experiments revealed that the mixed culture of strains B3 and B6 removed 84% of TPHs from soil polluted with 10% crude oil within 90 days. Although the mixed culture removed 28% of TPHs from soil contaminated with spent lubricating oil, the decrease in total abundance of *n*-alkanes was as high as 94%. The present data clearly suggest that both the selected bacterial strains (B3 and B6) could serve as potential candidates in oil spill bioremediation technology for the removal of PAHs and TPHs from different petroleum hydrocarbons.

Acknowledgements This work was financially supported by Secretaría Nacional de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT-Ecuador) Prometeo research project during 2013–2015.

References

Adesodun JK, Mbagwu JSC (2008) Biodegradation of waste-lubricating petroleum oil in a tropical alfisol as mediated by animal droppings. Bioresour Technol 99:5659–5665

Agarry SE, Ogunleye OO (2012) Factorial designs application to study enhanced bioremediation of soil artificially contaminated with weathered bonny light crude oil through biostimulation and Bioaugmentation strategy. J Environ Prot 3:748–759

Bhattacharya M, Biswas D, Sana S, Datta S (2015) Biodegradation of waste lubricants by a newly isolated *Ochrobactrum* sp. C1. 3. Biotech 5:807–817

Binazadeh M, Karimi IA, Li Z (2009) Fast biodegradation of long chain *n*-alkanes and crude oil at high concentrations with *Rhodococcus* sp. Moj-3449. Enzyme Microb Technol 45:195–202

Borah D, Yadav RNS (2014) Biodegradation of diesel, crude oil, kerosene and used engine oil by a newly isolated *Bacillus cereus* strain DRDU1 from an automobile engine in liquid culture. Arab J Sci Eng 39:5337–5345

Buccina S, Chene D, Gramlicha J (2013) Accounting for the environmental impacts of Texaco's operations in Ecuador: Chevron's contingent environmental liability disclosures. Accounting Forum 37:110–123

CIA World Factbook (2015) Economy overview. https://www.cia.gov/Library/publications/the-world-factbook/fields/2116.html. Accessed 16 Jan 2016

Das K, Mukherjee AK (2007) Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East India. Biores Technol 98:1339–1345

Gesinde AF, Agbo EB, Agho MO, Dike EFC (2008) Bioremediation of some Nigerian and Arabian crude oils by fungal isolates. Int Jor P App Scs 2:37–44

Gomaa EZ (2013) Biodegradation of polycyclic aromatic hydrocarbons by exopolymers synthesized by moderately halophilic bacteria: chemical composition and functional properties. J Polym Environ 21:495–503

Joo H, Ndegwa PM, Shoda M, Phae CG (2008) Bioremediation of oil-contaminated soil using *Candida catenulate* and food waste. Environ Pollut 156:891–896

Kang Y, Yong JP, Jaejoon J, Woojun P (2009) Inhibitory effect of aged petroleum hydrocarbons on the survival of inoculated microorganism in a crude-oil-contaminated site. J Microbiol Biotechnol 19:1672–1678

Koma D, Sakashita Y, Kubota K et al (2003) Degradation of car engine base oil by *Rhodococcus* sp. NDKK48 and *Gordonia* sp. NDKY76A. Biosci Biotechnol Biochem 67:1590–1593

Kostka JE, Prakash O, Overholt WA et al (2011) Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted by the deepwater horizon oil spill. Appl Environ Microbiol 77:7962–7974

Laila AF, Nour SEG (2008) Biodegradation of baleym mix crude oil in soil microcosm by some locally isolated Egyptian bacterial strains. Soil Sediment Contam 17:150–162

Larrea C, Warnars L (2009) Ecuador's Yasuni-ITT initiative: Avoiding emissions by keeping petroleum underground. Energy Sustain Dev 13:219–223

Laura F, Emilio R, Anthony SD, Angeles SM, Marta MP (2016) *Bacillus thuringiensis* a promising bacterium for degrading emerging pollutants. Proc Safe Environ 101:19–26

Lloyd C (2015) Oil and gas in Ecuador. Available from https://www.energyglobal.com/downstream/refining/23032015/eia-ecuador-oil-gas-491

Maddela NR, Masabanda M, Leiva-Mora M (2015) Novel diesel-oil degrading bacteria and fungi from Ecuadorian Amazon rainforest. Water Sci Technol 71:1554–1561

Maddela NR, Burgos R, Venkateswarlu K, Banganegiri M, Carrión AR (2016) Removal of crude oil from soil by using novel microorganisms of Ecuador soils: solid and slurry phase methods. Int Biodeter Biodegrad 108:85–90

Mukherjee AK, Bordoloi NK (2012) Biodegradation of benzene, toluene and xylene (BTX) in liquid culture and in soil by *Bacillus subtilis* and *Pseudomonas aeruginosa* strains and a formulated bacterial consortium. Environ Sci Pollut Res 19:3380–3388

Ogochukwu AB, Matthew OI, Simoncyril UN (2013) Utilization of drilling fluid base oil hydrocarbons by microorganisms isolated from diesel-polluted soil. Soil Sediment Contam 22:817–828

Rahman KSM, Banat IM, Thahira J, Thayumanavan T, Lakshmanaperumalsamy P (2002) Bioremediation of gasoline contaminated soil by a bacterial consortium amended with poultry litter, coil paith and rhamnolipid biosurfactant. Biores Technol 81:25–32

Rajasekar A, Ganesh Babu T, Karutha Pandian S, Maruthamuthu S, Palaniswamy N, Rajendran A (2007) Biodegradation and corrosion behavior of manganese oxidizer *Bacillus cereus* ACE4 in diesel transporting pipeline. Corrosion Sci 49:2694–2710

Ron EZ, Rosenberg E (2014) Enhanced bioremediation of oil spills in the sea. Curr Opin Biotechnol 27:191–194

Sathishkumar M, Binupriya AR, Baik SH, Yun SE (2008) Biodegradation of crude oil by individual bacterial strains and a mixed bacterial consortium isolated from hydrocarbon contaminated areas. Clean 36:92–96

Sebastian M, Hurtig AK (2004) Oil exploitation in the Amazon Basin of Ecuador: a public health emergency. Pan Am J Public Health 15:205–211

Supaphol S, Panichsakpatana S, Trakulnaleamsai S et al (2006) The selection of mixed microbial inocula in environmental biotechnology: example using petroleum contaminated tropical soils. J Microbiol Meth 65:432–441

US EPA (1996) Guidance on use of modeled results to demonstrate attainment of the ozone NAAQS, EPA-454/B-95-007, (file name: "O3TEST"), http://www.epa.gov/ttn/scram/

Venosa AD, Zhu X (2003) Biodegradation of crude oil contaminating marine shorelines and freshwater wetlands. Spill Sci Technol Bull 8:163–178

Verstraete W, Vanloocke R, Deborger R, Verlindne (1975) A modeling of the breakdown and the mobilizaiton of hydrocarbons in unsaturated soil layers. In: Sharpley JM, Kalpan AM (eds) Proceedings of 3rd international biodegradation symposium. Applied Science Publishers, London, pp 98–112

Walworth J, Pond A, Snape I et al (2007) Nitrogen requirements for maximizing petroleum bioremediation in a sub-Antarctic soil. Cold Reg Sci Technol 48:84–91

Yoon-Suk K, Park YJ, Jung J, Park W (2009) Inhibitory effect of aged petroleum hydrocarbons on the survival of inoculated microorganism in a crude-oil contaminated site. J Microbiol Biotechnol 19:1672–1678