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ORIGINAL PAPER

Assessment of bioremediation of aliphatic, aromatic, resin, and asphaltene fractions of oil-sludge-contaminated soil

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Abstract Oil sludge is a viscous material consisting of resin, asphaltene, sand, and water, which is usually formed at the bottom of oil reservoir tanks. Asphaltene and resin contents of oil sludge make it more resistant to biodegradation. Disposal of oil sludge is the main problem of oil industry; discharge of oil sludge into the soil causes damage to the environment. Bioremediation is an efficient, cheap, and environmentally friendly method for oil-sludge treatment. The aim of this study was to investigate the biodegradation of oil-sludge fractions in contaminated soil for a period of 12 months. The oil sludge was mixed with soil with the final concentration of 5 % (w/w), and the nutrients such as phosphate and nitrate salts were added to soil. Finally, reduction of aliphatic, aromatic, resin, and asphaltene was tested. The results showed that the reduction of total petroleum hydrocarbon was 31 ± 5 % during 12 months of the treatment. About 60 ± 8 % of total aliphatic fractions, mainly C_{14}–C_{22}, decreased. Analysis for the detection of two, three, and four rings of polycyclic aromatic hydrocarbons demonstrated about 42 ± 3 % reduction of total aromatic fractions, whereas the resin (6 ± 0.8 %) and asphaltene (4 ± 0.5 %) fractions were slightly biodegraded. In conclusion, biotreatment of oil sludge during 12 months could well reduce aliphatic and aromatic fractions, but more time is needed for resin and asphaltene reduction.

Keywords Biodegradation · Oil-sludge fractions · Polycyclic aromatic hydrocarbons · Soil contamination

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Introduction

Some parts of crude oil precipitate to the bottom of reservoirs and containers when stored in tanks or carried by ships. It cannot be drained from the tanks and must be removed at extensive costs In this regard, oil sludge is one of the main problems in oil refineries, since its entry into the environment can be harmful for the ecosystem.

Petroleum consists of various compounds which include aliphatic, aromatic, resin, and asphaltene (Colwell and Walker 1977). The petroleum industry produces large amounts of oily and viscous sediments. Many oil reservoir tanks and other containers in petroleum-processing factories contain precipitated sediments, which are collected over time. These residues, called oily sludge, are composed of oil, water, solids, and compounds such as high content of resin and asphaltene, which make them highly recalcitrant and very difficult for re-utilization. Oil sludge at the bottom of tanks can promote corrosion and reduce storage capacity. During cleaning operations, oil sludge is removed and dumped in nearby lands. This sediment is recalcitrant, and if left in the soil for many years, it will harm the environment. Some methods such as incineration are used to eliminate oil sludge from the environment, but this method is expensive and produces a variety of toxic compounds to the air. Landfill may be also proposed for oil-sludge disposal; however, oil sludge buried in the soil can affect its physicochemical properties and also harm its living organisms. Bioremediation is the best method for the removal of oil pollution from the soil. Some oil-degrading bacteria are able to degrade oil and use it as a carbon source for their growth (Leahy and Colwell 1990; Vasudevan and Rajaram 2001). Some petroleum components are toxic for living organisms; however, some plants and microorganisms are able to biodegrade the crude oil

hydrocarbons into less toxic products than the parent compounds (Eweis et al. 1998). The effect of contaminant on microorganisms and plants depends on the concentration and kind of contamination (Boethling and Alexander 1979; Minai-Tehrani 2008). Many environmental parameters can affect the bioremediation of oil sludge, which include pH of soil, nutrients, moisture, and aeration (Dibble and Bartha 1979; Ururahy et al. 1998). The amount of nitrate and phosphate is important for biodegradation of oil-contaminated soil and water (Zekker et al. 2011; Zekker et al. 2012a). Potent bacterial consortium and biosurfactant have been also useful for the bioremediation of petroleum and oil sludge (Bordoloi and Konwar 2009; Cameotra and Singh 2008). As oil sludge has higher resin and asphaltene than crude oil, it cannot be well degraded by microorganisms and plants and remains in the soil for many years (Walker et al. 1975). It has been shown that the rate of bioremediation is maximum in saturated aliphatic and light aromatic fractions of petroleum (Jobson et al. 1972; Walker et al. 1976). Although there are few reports on the biodegradation of oil sludge in the soil (Dibble and Bartha 1979; Ururahy et al. 1998; Vasudevan and Rajaram 2001), none of them has reported the biodegradation of oil sludge and its fractions in detail. This study focused on the bioremediation of various fractions such as aliphatics, aromatics, resins, and asphaltene of oil sludge in the soil. Oxygen, nutrients, and moisture which are essential components for well-biodegraded oil-contaminated soils (Braddock et al. 1997; Dibble and Bartha 1979; Zekker et al. 2012b) were considered in this study. Reduction of aliphatic and aromatic fractions was also analyzed.

Materials and methods

Soil preparation

Cultivation soil was acquired from an area near Tehran Refinery (capital of Iran) and dried at 50 °C for 48 h; then, large particles were removed by sieving with 4 mm mesh. Characteristics of the soil used in this experiment are indicated in Table 1. Hydrometer and Walkley–Black methods were used to obtain the soil texture and its organic matter, respectively (Bouyoucos 1962; Gee and Bauder 1986; Robertson et al. 1999). The oil sludge was obtained from the Maroon oil reservoir tank in the northwest of Persian Gulf and added to 500 g of the soil with the concentration of 5 % (w/w). For mixing oil sludge with the soil, the mixture was transferred in the 1-kg bucket.

After determining the water-holding capacity (field capacity) of the soil, moisture of the soil was kept at 60–65 % of field capacity using tap water (Dibble and Bartha 1979). The soil was mixed every other day, thereby inducing aeration in the buckets. The aeration was done once by mixing the soil and by turning the whole soil upside down within the pails. Addition of water and aeration was done for a period of 12 months in laboratory with constant temperature about 28–30 °C.

Table 1 Soil characteristics used in this study

Soil parameters	
Clay	31 %
Silt	56 %
Sand	13 %
Organic matter	3.1 %
Calcium carbonate	26 %
pH	7.1
Total *N*	0.03 %
Total *P*	6 ppm
K	19.5 ppm
Mg	16 ppm
EC (electrical conductivity)	4.3 dS/m

The control sample (three replicates) had the same conditions as the aforementioned samples, but no aeration and water were applied.

Colony count

The number of microorganism colonies in the soil was resolved by *pure-plate* method (Cappuccino and Sherman 1996) every 6 months and compared with time zero (start). About 1 g of soil was removed from each sample and dissolved in normal saline solution (NaCl 0/9 %), and serial dilutions were prepared for each sample. Nutrient agar containing plate was used for counting the colonies. The plates were incubated at 30 °C for 48 h and then counted for the number of colonies (total colonies). To resolve the number of oil-degrading bacteria, the diluted samples were transferred to the plates containing agar-agar mixed in salt solution (NH_4NO_3 1 g, Na_2HPO_4 2.5 g, and KH_2PO_4 2.5 g per liter with pH 7) and crude oil (1 %) as the only source of carbon. The incubation condition was the same as that for total bacterial count.

Total petroleum hydrocarbon (TPH) extraction

At the end of the experiment (12 months), the amount of TPH in the contaminated soils was determined according to the method used by Minai-Tehrani and Herfatmanesh (2007). From each sample, 1 g of soil was removed and dried at 50 °C. The dried soils were crushed to obtain homogenous samples; then, it was dissolved in 10 ml of dichloromethane (CH_2Cl_2 Merck) while firmly shaking the mixture to separate oil from the soil. To precipitate the soil,

the mixture was centrifuged and the supernatant which contained the solvent phase was removed.

The extraction procedure was repeated twice. The solvent was vaporized during 24 h, the remaining oil was weighed by gravimetric method, and the reduction was compared to the start time. From each replicate, two samples were taken for oil extraction.

Fractionation of oil

Separation of aliphatic, aromatic, resins, and asphaltene was approved by SARA method (saturate, aromatic, resin, and asphaltene (SARA) is an analysis method that divides crude oil components according to their polarizability and polarity) (Speight 2004; Vasquez and Mansoori 2000). After extracting TPH by the aforementioned method, the residue was dissolved in 10 ml n-hexane (Merck) and filtered to separate the insoluble asphaltene. The insoluble residue was weighed to determine the amount of asphaltene. The filtered hexane extract was loaded to a 1 × 25 cm column packed with 20 cm silica gel and 5 cm Na_2SO_4. The column was pre-washed with n-hexane. About 30 ml of n-hexane was used as the mobile phase to release the aliphatic fractions. After solvent evaporation, the residue was weighed to determine total aliphatic fractions, and then, the residue was dissolved in n-hexane for gas chromatography (GC) analysis. About 1 µl of the sample was injected into gas chromatograph (Hewlett-Packard HP 5890A) equipped with FID detector and fused silica capillary column. The carrier gas was H_2 with the injection temperature of 300 °C, while that of the detector was 330 °C.

To release aromatic fractions from the column, 30 ml of n-hexane/dichloromethane (1:1, v/v) was used, the aromatics were collected, and the solvent was evaporated. The residue was weighed to determine the total amount of aromatic fraction of each sample. The residue was dissolved in 5 ml acetonitrile for high-performance liquid chromatography (HPLC) analysis. About 15 µl of the sample was injected into HPLC column, with water/acetonitrile (1:2, v/v) as the mobile phase and flow rate of 1 ml/min equipped with a UV detector at 254 nm. Some polycyclic aromatic hydrocarbon (PAH) such as naphthalene, acenaphthylene, phenanthrene, anthracene, fluoranthene, pyrene, and chrysene was prepared as the standard (Supelco mix PAH standard) and injected into the HPLC column (Shimadzu LC 10A HPLC system equipped with a C_{18} column). Retention time of each compound and their exiting regions from the column were used to localize them in main graphs of the samples.

Finally, to release the resins from the column, 50 ml of toluene/methanol (60/40 v/v) was used. After evaporation of the solvent, the amount of resins was determined by gravimetry.

Statistical analysis

Results were expressed as mean ± standard deviation (±SD) and analysis of variance, and statistically significant difference ($p < 0.05$) was done by one-way ANOVA test. Comparison of the means to obtain significant differences was performed by Tukey test. The statistical results were analyzed by GraphPad Prism 5 program.

Results and discussion

Colony count

Determination of total and oil-degrading microorganisms showed that the number of colonies increased in the treated soil (Fig. 1). A significant difference was observed in the counts of both 6 and 12 months with that of the start time. The difference was not significant between 6 and 12 months, neither in total nor in oil-degrading microorganisms. However, total and oil-degrading counts after 6 months were higher than those after 12 months of treatment. The number of total and oil-degrading microorganisms increased in the treated soil, which suggested that the biodegradation occurred in the contaminated soil. Although the concentration of oil sludge (5 % w/w) was toxic and harmful for the microorganisms, the number of microbial colonies increased in the soil. Previous reports have also demonstrated that, in crude oil-contaminated soil, the number of microbial colonies increased by increasing the concentration of crude oil (Chorom et al. 2012; Minai-Tehrani et al. 2012). Minor colony reduction was observed after 12 months of treatment in comparison

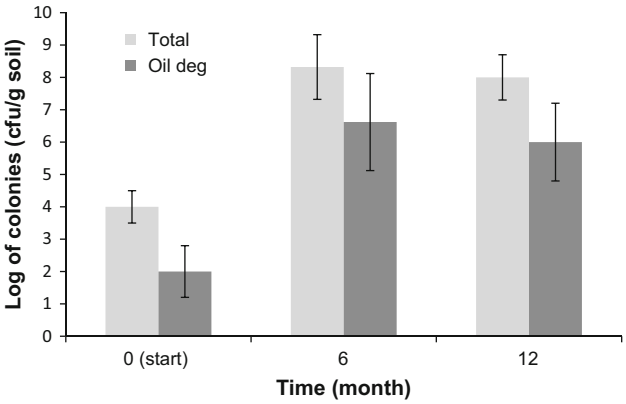

Fig. 1 Determination of total and oil-degrading colony counts after 6 and 12 months of treatment. The number of colonies was reduced in the control sample after 6 and 12 months but the difference was not significant with start time (data not shown). Average values given ±SD ($n = 3$)

with 6 months, representing that most biodegradable hydrocarbons might be consumed in the first 6 months of treatment and the remaining hydrocarbons including heavy aromatics, resin, and asphaltene were not easily degraded by the microorganisms. This issue could be the reason for the reduction of colonies in the second 6 months of treatment.

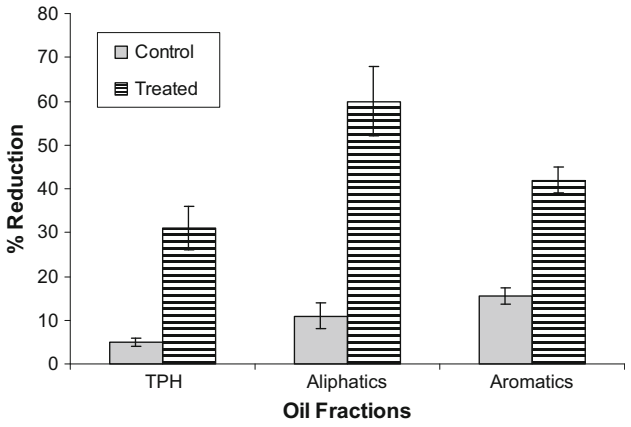

Fig. 2 Reduction of TPH, total aliphatic and aromatic fraction of oil sludge after 12 months of treatment. The significant difference was observed between the control and treated samples ($n = 3$, $p < 0.05$)

Total TPH reduction

Reduction of total petroleum hydrocarbon (TPH) and total aliphatic and aromatic fractions was measured after 12 months (Fig. 2). The reduction was considered as a percent of reduction by comparing the amount of fractions at the end of the experiment and the start time (time = 0). In all the cases, a significant difference was observed between the control group and treated samples. Reduction of total aliphatic fractions was higher than that of the total aromatic fractions with a significant difference.

Aliphatic reduction

After 12 months ($T = 12$) of treatment, the soil was analyzed in terms of the degradation of particular aliphatic chain length. GC analysis was performed for determining details of aliphatic reduction (Fig. 3) in the control and

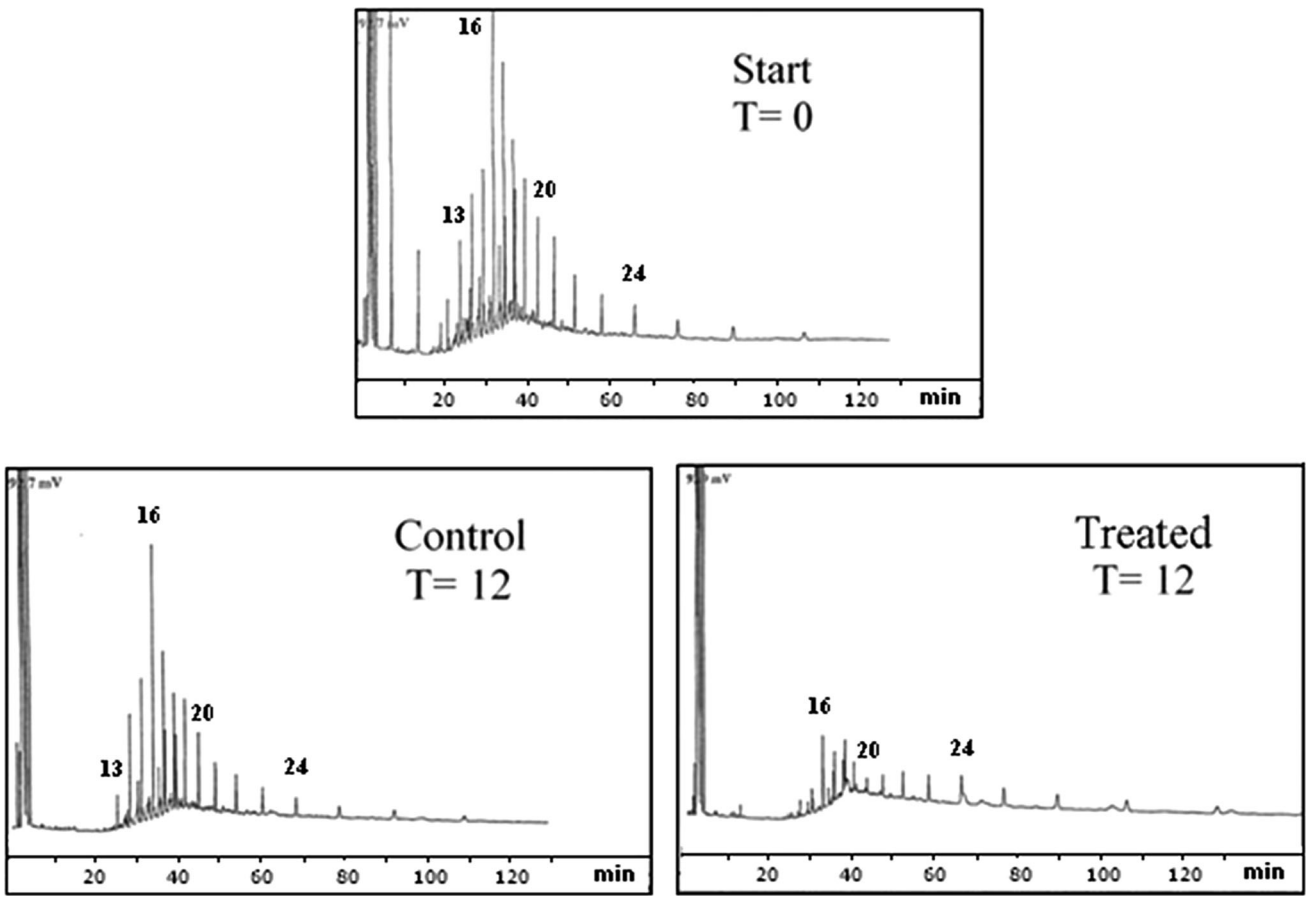

Fig. 3 GC graphs from aliphatic fraction of oil sludge after 12 months of treatment ($T = 12$). The control and treated samples were compared with the aliphatic fraction of soil at start time ($T = 0$). The *digits* over the peaks indicate the number of carbon

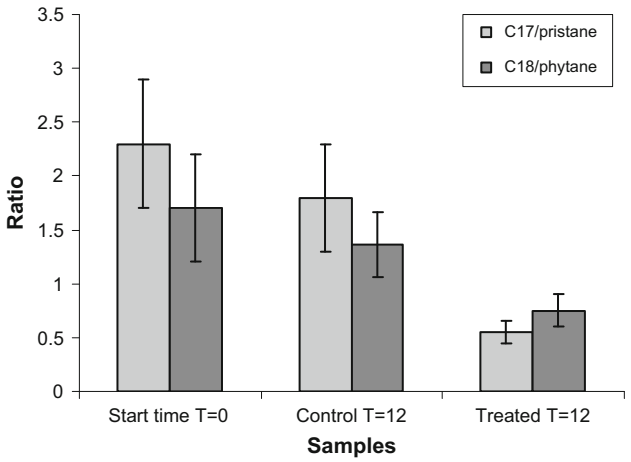

Fig. 4 Ratios of C_{17}/phytane and C_{18}/pristine in the control and treated samples after 12 months compared with start time. These ratios were lower than 1 in the treated sample while they were higher than 1 in the control group. Average values given ±SD ($n = 3$)

treated samples and comparing with the start time ($T = 0$). Reduction of the treated sample was higher than that of the control. Most of the eliminated aliphatics were between C_{12} and C_{22} hydrocarbons.

Reduction ratios of normal C_{17} and C_{18} alkanes to their branched isomers, pristane, and phytane are usually used as indices of biodegradation and for monitoring biological effect on the aliphatic fractions of oil. In the treated sample, these ratios fall to below one (Fig. 4), while they are bigger than one in the control group. Earlier reports have demonstrated that aliphatic fractions are biodegraded with higher efficiency than aromatic fractions, which determine that the aliphatics can be better consumed by bacteria (Jobson et al. 1972; Juteau et al. 2003; Minai-Tehrani and Herfatmanesh 2007). Present results also showed that the aliphatic fractions were the most degradable components of the oil sludge. Gas chromatography (GC) analysis indicated that, among the aliphatics, carbon fractions of 14–22 had higher reduction. The control group was considered for this experiment to find out the role of volatilization in the reduction of oil sludge and its fractions. Previous reports have shown that both volatilization and biodegradation are important for aliphatic and aromatic fractions of crude oil (Leahy and Colwell 1990; Nicodem et al. 1997). Biodegradation changes both straight chain and branched aliphatics; however, branched isomers biodegrade more slowly than straight chains hydrocarbons (Atlas 1975) and the reduction ratios of normal C_{17} and C_{18} alkanes to their branched isomers, pristane, and phytane are usually used to study the effect of volatilization and biodegradation of aliphatic fractions (Minai-Tehrani and Herfatmanesh 2007; Seklemova et al. 2001). Present experiment revealed that the low ratio of C_{17}/pristane and C_{18}/phytane in the treated sample indicated the main role of biodegradation in aliphatic reduction, while the high ratios of these parameters in the control group point to the volatilization had the major role in aliphatic reduction.

Aromatic reduction

To determine the reduction of polycyclic aromatic hydrocarbons (PAHs), HPLC analysis was performed. At the end of the experiment (12 months), the PAH content of the oil-sludge-contaminated soil of the control and treated samples was compared to the start time ($T = 0$) (Fig. 5). In the treated samples, higher reduction of PAHs was observed in comparison with the control. All the PAHs, from two to four rings, were decreased in the treated samples with a significant difference compared to the control (Fig. 6). The two-ring PAHs such as naphthalene and acenaphthylene had higher reduction in the control group than the three- and four-ring PAHs. Present results showed that the aromatic fractions were also well biodegraded by the bacteria. These results were in accordance with the previous reports indicating that aliphatic fractions are biodegraded with higher efficiency in crude oil-contaminated soil (Minai-Tehrani et al. 2006; Minai-Tehrani and Herfatmanesh 2007). HPLC analysis was performed to determine the pattern of PAHs elimination from the soil. Figure 4 shows that volatilization had a major function in the reduction of two-ring PAHs such as naphthalene and acenaphthylene. Conversely, biodegradation has played the main role to reduce the three- and four-ring PAHs. The three-ring PAHs biodegraded with higher efficiency than the four-ring PAHs.

Other reports have also represented that the two- and three-ring PAHs are biodegraded with higher efficiency than four- and five-ring PAHs from crude oil-contaminated soil (Minai-Tehrani et al. 2009; Minoui and Minai-Tehrani 2009).

Resins and asphaltene

Reduction of resin and asphaltene was investigated in the treated samples and compared to the control (Fig. 7). A significant difference was observed between the treated samples and control group for the reduction of both resins

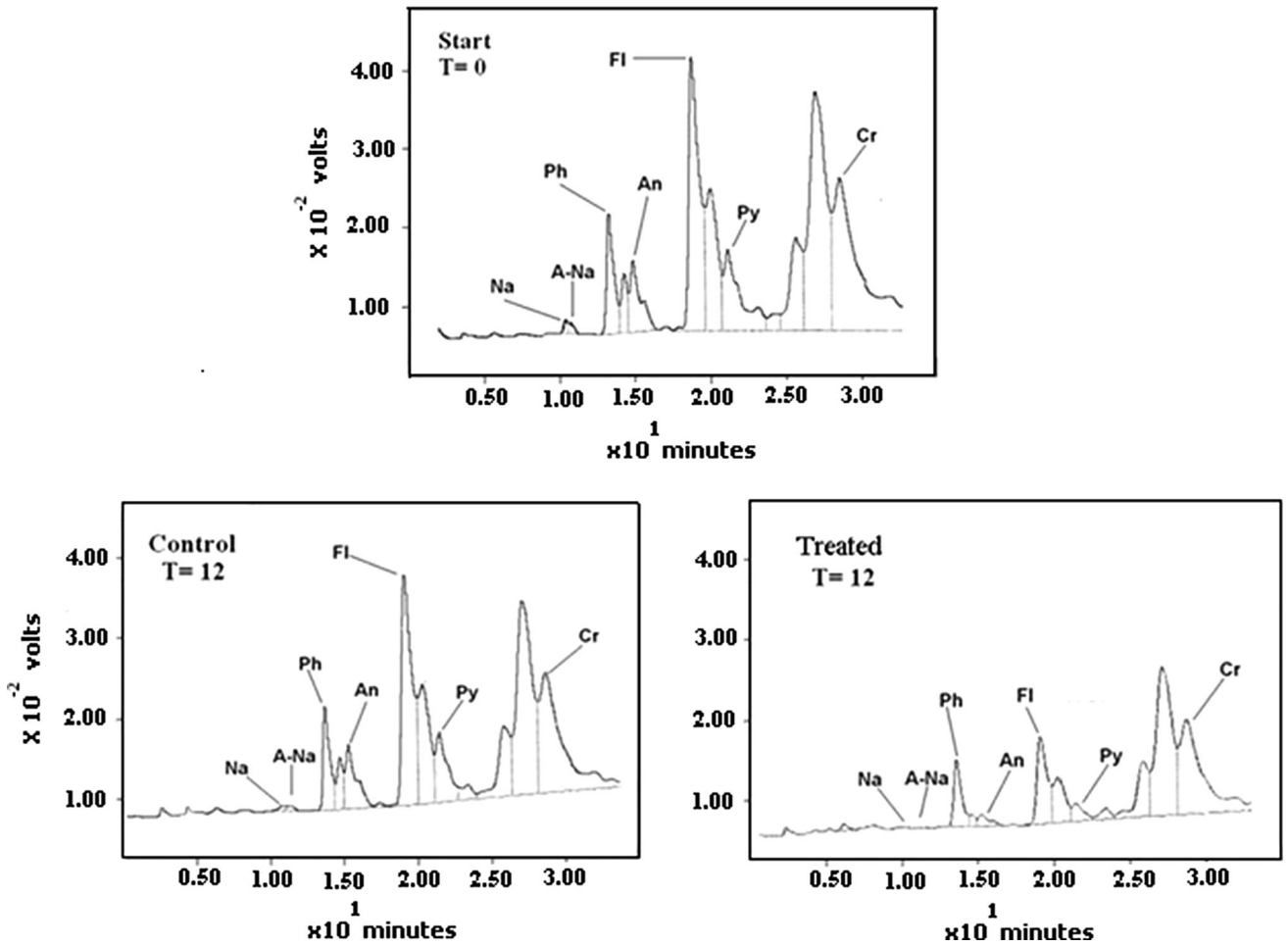

Fig. 5 HPLC graphs of aromatic fractions of oil sludge after 12 months of treatment ($T = 12$). The reduction of PAHs in the control and treated sample was compared with the start time ($T = 0$). *Na* naphthalene, *A-Na* acenaphthylene, *Ph* phenanthrene, *An* anthracene, *Fl* fluoranthene, *Py* pyrene, *Cr* chrysene

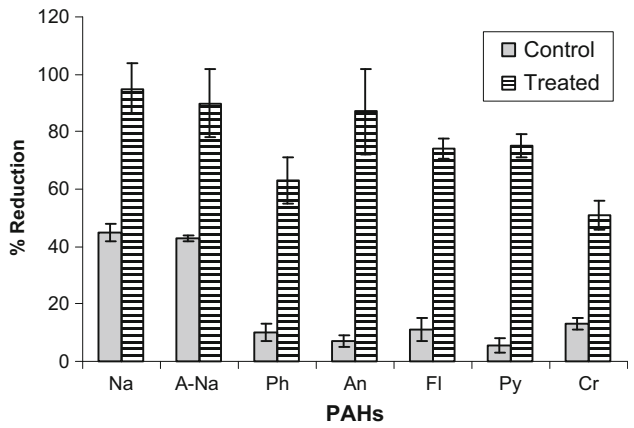

Fig. 6 Reduction of different PAHs after 12 months. *Na* naphthalene, *A-Na* acenaphthylene, *Ph* phenanthrene, *An* anthracene, *Fl* fluoranthene, *Py* pyrene, *Cr* chrysene. In all tested PAHs, a significant difference was observed between the control and treated samples ($n = 3$, $p < 0.05$)

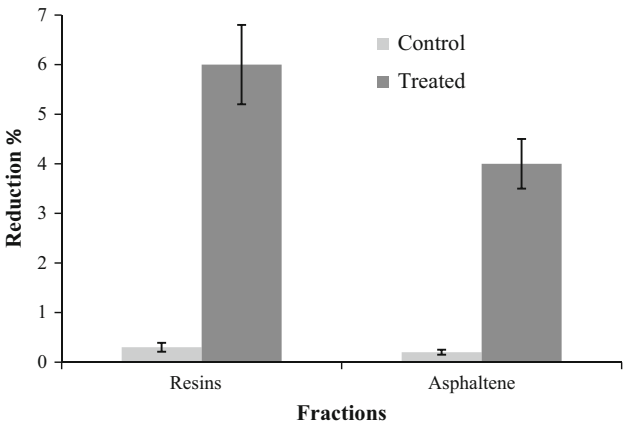

Fig. 7 Resin and asphaltene reduction after 12 months of soil treatment. Average values given ±SD ($n = 3$, $p < 0.05$)

and asphaltene. Reduction of resins was more, but not notably, than asphaltene. Although the resins and asphaltene seem to be resistant to biodegradation, these results showed that both were biodegraded at a slow rate after 12 months of treatment.

Conclusion

In this study, biodegradation of various oil-sludge fractions was analyzed. It has been shown that viscous oil sludge consisting of recalcitrant compounds could biodegrade in the soil. The most degradable fractions of oil sludge were aliphatic and aromatic fractions. Resin and asphaltene were degraded at a very slow rate.

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