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ORIGINAL PAPER

Application of indigenous microbial consortia in bioremediation of oil-contaminated soils

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Abstract Bioremediation of oil spillage in soils using consortia of microbes beckons much exploration. The present study involves bioremediation of oil-contaminated soils from north Chennai, India, using indigenous microbial consortia. Totally, 32 positive oil degrading isolates were obtained from 3 different locations, i.e., petrol filling stations, automobile workshops and oil refineries. Substrate utilization patterns of individual isolates and the consortial sets were observed. Mixture of three common hydrocarbons (petrol, diesel and engine oil) was used for studies. The substrate oil utilized by consortia was taken for thin-layer and column chromatography which perfectly resulted in varied fractions of oil compared to the unused oil as control. The best consortia were used directly for bioremediation experiment. Three different oil-contaminated soils were used and bioremediation patterns were observed. The rate of bioremediation differed within soils, nevertheless all soils were almost 100 % reclaimed within 30 days. Bioremediation kinetics showed that the process corresponds to first-order kinetics and kinetic constants for the different soils ranged from 0.051 to 0.077/day. Assessment of detoxification of acute phytotoxicity owing to the pollutant oil was done, and results observed were significant. An increase of 25, 300 and 212 % in germination index, average growth index and sustenance index, respectively, of *Trigonella foenum-graecum* Linn. in treated soils was observed, compared to untreated soils. Thus, this study confirmed that microbes in 'Consortial Union' serve as better treating agents in bioremediation of oil-contaminated soils than individual microorganisms.

Keywords *Trigonella foenum-graecum* · Hydrocarbons · Kinetics · Phytotoxicity

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Introduction

Petroleum and its products proved to be one of the most beneficial materials are now becoming a drawback since the complex hydrocarbon makeup of these products is causing serious pollutions. Crude oil is a complex mixture of thousand of compounds that can potentially be degraded by a great variety of soil and aquatic microorganisms (Radwan and Sorkhoh 1993). Crude oil can be accidentally or deliberately released into the environment leading to serious pollution problems (Thouand et al. 1999). The release of hydrocarbons into the environment, whether accidental or due to human activities, is the main cause for water and soil pollution (Boehm et al. 1995; Abdulsalam et al. 2011). Many bioremediation technologies have been developed to remove these contaminants, as some biological treatments are cheaper than chemical and physical treatments and sometimes result in complete mineralization (Holliger et al. 1997). Several technologies have been adapted to restore the harmony of nature by reclaiming the soils and seas contaminated by such undesired spillages. However, the hunt for cheaper and faster

processes is always been done to create a significant progress. One such technology is using indigenous microorganisms to degrade the hydrocarbon pollutants and remediate the lost soil parameters.

Bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes (Vidali 2001).

Indigenous microorganisms can utilize the total petroleum hydrocarbons of crude oil as source of carbon and energy and break them down to simpler non-toxic compounds such as CO2 and H2O. The bacterial groups known to degrade hydrocarbons include *Pseudomonas, Marinobacter*, *Alcanivorax*, *Microbulbifer*, *Sphingomonas*, *Micrococcus*, *Cellulomonas*, *Dietzia* and *Gordonia*groups (Brito et al. 2006). Molds belonging to the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Amorphoteca*, *Neosartorya*, *Paecilomyces*, *Talaromyces*, *Graphium* and the yeasts *Candida*, *Yarrowia* and *Pichia* have been implicated in hydrocarbon degradation (Chaillana et al. 2004).

Also, microbes used recently for bioremediation processes are usually consortia as different microbial strains having the same function can actually create synergistic effects and treat the experimented site sooner than expected. Possibility of one species removing the toxic metabolites (that otherwise may hinder microbial activities) of the species preceding it. It is also possible that the second species are able to degrade compounds that the first are able to only partially degrade (Alexander 1999). Therefore, the present study also focuses bioremediation of contaminated sites using microbial consortia.

The complete objective for any bioremediation study is to finally restore the natural property of soil that has been contaminated before. Usually, hydrocarbon contamination leads to loss of soil fertility. Therefore, ensuring that the remediation experiment has actually restored back the property of soil to support plant growth becomes important. This study is also called as toxicity analysis (Millioli et al. 2009).

There are several types of toxicity studies involving plant processes. Tests with plants can be used in 5 different categories: biotransformation, food chain uptake, sentinel, surrogate and phytotoxicity. Among those tests, the phytotoxicity is receiving more attention during the last years (Fletcher 1991). Generally, any plant having very quick germination and growth cycle can be used for phytotoxicity analysis. Germination studies are considered short-term and primarily assess acute toxicity effects. In germination studies, seeds are planted in a small quantity of the contaminated soil, and seedlings counted after an extended incubation period. Results are compared to seedling enumeration in uncontaminated control soil. Seed germination has been shown to decrease significantly in heavy metal and hazardous contaminated soil. Plants that are sensitive to poisonous substances can be used as bio indicators (Banks and Schultz 2005).

The present study deals with the isolation of indigenous microbes from the oil-contaminated soils, determination of its potential in various hydrocarbons degradation and its application in bioremediation of soil fertility. The present study was carried out during December 2010 to June 2011 in Life Teck Research Centre, Chennai, India.

Materials and methods

Isolation of microbes from oil-contaminated samples

Oil-contaminated soil samples were collected from different places like petrol filling stations, automobile workshops in and around Vadapalani, Chennai, and oil refinery area from Manali, Chennai. One gram of soil sample was serially diluted using sterile saline, and 0.1 mL was spread in the sterile nutrient agar plates. The plates were incubated at 37 °C for 24 h. Isolates with different colony morphology were sub cultured in nutrient agar slants and used for further investigations. The isolates were identified using a battery of biochemical characteristics as described in Bergey's manual.

Screening for oil degraders

Bushnell Haas broth (BHB) was prepared with 10 % of oil substrates (Mandri and Lin 2007). The oil substrates were petrol, diesel and engine oil. The isolates were enriched in BHB for 48 h at 37 °C. All isolates from BHB were plated in Bushnell Haas agar (BHA) with 100 µl of one of the respective oil substrates (petroleum, diesel and engine oil). Plates were incubated at 37 °C for 1 week. Positive isolates were sub cultured and used for further studies.

Substrate utilization of individual isolates

All positive isolates were independently inoculated in BHB containing either all three or two out of three substrates

alternatively. The isolates were kept in mechanical shaking at 100 rpm at 37 °C for 7 days. Aliquots of growth medium were taken at an interval of 24 h, and absorbance was measured at 600 nm using UV–VIS Spectrophotometer (Shimadzu® UVmini-1240). Uninoculated medium was taken as blank.

Consortial preparation

Only positive isolates from above experiment on BHB media containing all three substrates were taken for consortial preparation. Four sets (A, B, C, D) were made based on possible capability of organisms to degrade the substrates. Organisms of different genera which utilized similar kind of oil substrates were selected and mixed in equal amounts.

Substrate utilization of consortia

The organisms selected for consortia were mixed aseptically in a separate tube in equal amounts. 100 µl from this mixture was inoculated in separate tubes containing BHB with all three substrates and kept in mechanical shaker (REMI® RIS-24 BL) at 100 rpm at 37 °C for 7 days. Aliquots of growth medium were taken, and absorbance was measured at 600 nm as specified earlier. Uninoculated medium was taken as blank.

Determination of biosurfactant activity

Drop collapse method was performed (Tugrul and Cansunar 2005). Culture was centrifuged (REMI® C-24 BL) at 10,000 rpm for 10 min and supernatant was collected. Three drops of engine oil were placed over a clean glass slide, and 10 µl of the supernatant was added over one of the oil films. Distilled water and Tween 80 were taken as negative and positive controls, respectively.

Thin-layer chromatography (TLC)

The oil used as substrate for microbial growth was extracted using solvent extraction method (Chang 1998; Marquez-Rocha et al. 2001) and was dissolved in 1 ml chloroform and spotted on silica gel F_{254} TLC plates (Merck®).The chromatogram was developed with benzene, petroleum ether and methanol (1:1.5:1) in the pre-saturated chromatographic chamber. The TLC plate was sprayed with 5 % w/v of Vanillin in Conc. Sulfuric acid and heated at 90–100 °C in hot air oven for 2–5 min. Commercial engine oil was taken as control.

Column chromatography

Column chromatography was done to analyze the fragments of substrate oil (Mittal and Singh 2009). Oil used as substrate for microbial growth was extracted as before after 10 days of microbial growth from the growth media. Pure oil was used as control. The chromatographic glass column (Borosil®, Internal diameter 2 cm, length 45 cm and reservoir capacity 80–100 ml) was packed with silica gel (60–120 mesh) and alumina pre-activated at 150 °C for 24 h. The slurry of 20 g activated alumina was packed in the column; followed by 20 g silica gel. The column was equilibrated with petroleum ether. The extracted oil sample was dissolved in chloroform and charged into the column. The elutes were collected using a series of solvents such as 60 ml of petroleum ether (40°–60 °C), 90 ml benzene and 60 ml methanol, respectively. The elutes collected were concentrated to dryness at 60 °C and each fraction was then weighed separately.

Bioremediation studies

Three different soils (200 g) were collected from highly oil-contaminated regions around Manali area, Chennai, and 10 % (v/w) of microbial consortia were inoculated over the soils and kept at room temperature for couple of weeks. Samples were taken once in 10 days for gravimetric analysis, and the amount of oil residues was determined. Soils without loaded microbial cultures were kept as control. The broth containing the microbial consortia was diluted to concentration of 10^2–10^3 colony forming units and applied over soils. The set D used in substrate utilization studies was directly used for bioremediation studies.

Determination of oil degradation

The level of oil degradation in soils was determined using the gravimetric analysis. (Chang 1998; Marquez-Rocha et al. 2001). Percentage of the remaining oil was calculated compared to the uninoculated soil (control).

Plantation studies

Growth and sustenance of plants were tested in soils (Millioli et al. 2009). Treated soil from above experiment and untreated soil were taken and *Trigonella foenum-graecum* Linn. (fenugreek, Family-Fabaceae) was sown and water was sprinkled routinely. Germination and growth

were observed. The following parameters were calculated as mentioned.

- Germination Index—number of seeds germinated/total seeds sown.
- Growth Index—length of stem/total height of plant.
- Sustenance Index—number of plantlets germinated/number of plantlets remaining at the end of one growth cycle.

Results and discussion

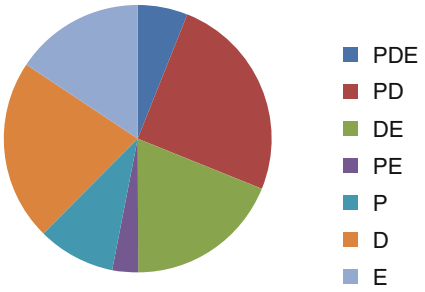
Screening for oil degraders

Among the three soil samples tested, a total of 49 isolates were isolated on nutrient agar plate.

The isolates were characterized as *Bacillus* sp. and *Pseudomonas* sp. based on series of biochemical tests shown in Table 1. Among the 49 isolates, 65.30 % (32) isolates were able to degrade the oil substrates and grow in the medium with oil as the sole carbon source. Among the positives isolates, 56.25 % (18) and 40.62 % (14) were found to be *Bacillus* sp. and *Pseudomonas* sp., respectively (Fig. 1). The ability of the isolates to degrade the different oil substrates was shown in Table 2. Hydrocarbon degrading bacteria and fungi are widely distributed in marine, freshwater and soil habitats. Similarly, hydrocarbon degrading cyanobacteria have been reported (Lliros et al. 2003).

Table 1 Biochemical characteristics of the positive isolates from oil-contaminated soils

Tests	*Bacillus* sp.	*Pseudomonas* sp.
Colony morphology	Opaque, raised, irregular and grayish white in color	Transparent irregular bluish green in color.
Gram reaction	Positive	Negative
Cellular morphology	Rods	Rods
Motility test	Motile	Motile
Indole	Negative	Negative
Methyl red	Negative	Negative
Voges proskauer	Negative	Negative
Citrate utilization	Negative	Positive
Catalase	Positive	Positive
Oxidase	Negative	Positive
Urease	Negative	Negative
Triple sugar iron agar	Acid butt alkaline slant	Alkaline butt alkaline slant hydrogen sulfide production



PDE - 6.25%, PD - 25.8%, DE - 19.35%, PE - 3.22%, P -9.67%, D - 22.58%, E -16.12%.

Fig. 1 Distribution of oil utilizing positive isolates

Substrate utilization of individual isolates

All the positive isolates were inoculated in two sets of BHB, one containing all three substrates and other containing two of three substrates alternatively. This was made to ensure whether the organisms had capability to degrade mixed substrates. Observations made it clear that some organisms which utilized the substrates individually were unable to utilize mixed substrates as depicted in Table 3. During bioremediation, microbes utilize chemical contaminants in the soil as an energy source and, through oxidation–reduction reactions, metabolize the target contaminant into useable energy for microbes. By-products (metabolites) released back into the environment are typically in a less toxic form than the parent contaminants. For example, petroleum hydrocarbons can be degraded by microorganisms in the presence of oxygen through aerobic respiration. The hydrocarbon loses electrons and is oxidized while oxygen gains electrons and is reduced. The result is formation of carbon dioxide and water (Nester et al. 2001).

Consortia preparation

Consortia of positive isolates containing *Pseudomonas* sp. and *Bacillus* sp. were done as in Table 4. Also, microbes used in modern studies for bioremediation processes are usually consortia as different microbial strains having the same function can actually create synergistic effects and treat the experimented site sooner than expected. Petroleum biodegradation has been reported to be mostly enhanced in the presence of a consortium of bacterial species compared to monospecies activities (Ghazali et al. 2004).

Table 2 Positive isolates from BHA plates which used oil as the sole carbon source

S. no	Isolate	Type of species	Petrol (P)	Diesel (D)	Engine oil (E)
1	P1	*Pseudomonas* sp.	+	+	+
2	B1	*Bacillus* sp.	+	+	+
3	P2	*Pseudomonas* sp.	+	+	–
4	P3	*Pseudomonas* sp.	+	+	–
5	P4	*Pseudomonas* sp.	+	+	–
6	P5	*Pseudomonas* sp.	+	+	–
7	P6	*Pseudomonas* sp.	+	+	–
8	P7	*Pseudomonas* sp.	+	+	–
9	B2	*Bacillus* sp.	+	+	–
10	B3	*Bacillus* sp.	+	+	–
11	P8	*Pseudomonas* sp.	–	+	+
12	P9	*Pseudomonas* sp.	–	+	+
13	P10	*Pseudomonas* sp.	–	+	+
14	B4	*Bacillus* sp.	–	+	+
15	B5	*Bacillus* sp.	–	+	+
16	B6	*Bacillus* sp.	–	+	+
17	P11	*Pseudomonas* sp.	+	–	+
18	P12	*Pseudomonas* sp.	+	–	–
19	B7	*Bacillus* sp.	+	–	–
20	B8	*Bacillus* sp.	+	–	–
21	P13	*Pseudomonas* sp.	–	+	–
22	P14	*Pseudomonas* sp.	–	+	–
23	B9	*Bacillus* sp.	–	+	–
24	B10	*Bacillus* sp.	–	+	–
25	B11	*Bacillus* sp.	–	+	–
26	B12	*Bacillus* sp.	–	+	–
27	B13	*Bacillus* sp.	–	+	–
28	P15	*Pseudomonas* sp.	–	–	+
29	P16	*Pseudomonas* sp.	–	–	+
30	P17	*Pseudomonas* sp.	–	–	+
31	B14	*Bacillus* sp.	–	–	+
32	B15	*Bacillus* sp.	–	–	+

Substrate utilization studies

All the isolates were individually found for their capability in degrading each one of the provided substrates. Then, the synergistic effect of mixed populations of so called 'consortia' was analyzed (Fig. 2).

Drop collapse method

It was observed that the drop got flattened on application of the culture supernatants and the drop remained as bead when water is used as control. A drop of water applied to a hydrophobic surface in the absence of surfactants will form a bead. The bead forms because the

Table 3 Positive isolates on mixed substrates

S. no	Isolate	PDE	PD	DE	PE
1	P1	+	+	+	+
2	B1	+	+	+	+
3	P2	+	+	+	+
4	P3	+	+	+	+
5	P4	–	+	–	–
6	P5	+	+	+	+
7	P6	+	+	+	+
8	P7	+	+	+	+
9	B2	+	+	+	+
10	B3	–	+	–	–
11	P8	+	+	+	+
12	P9	+	+	+	+
13	P10	–	–	+	–
14	B4	–	–	+	–
15	B5	–	–	+	–
16	B6	+	+	+	–
17	P11	–	–	–	–
18	P12	+	+	–	+
19	B7	+	+	–	+
20	B8	–	–	–	–
21	P13	+	+	+	–
22	P14	+	+	+	–
23	B9	+	+	+	–
24	B10	–	–	–	–
25	B11	+	+	+	–
26	B12	+	+	+	–
27	B13	+	+	+	–
28	P15	–	–	–	–
29	P16	–	–	–	–
30	P17	–	–	–	–
31	B14	+	–	+	+
32	B15	+	–	+	+

Table 4 Consortia selection

Sets	Isolates
A	P1, B1
B	P2, P3, P5, P6, P7, P8, P9, B2, B6
C	P12, P13, P14, B7, B9, B11, B12, B13, B14, B15
D	All isolates used in sets A, B, C

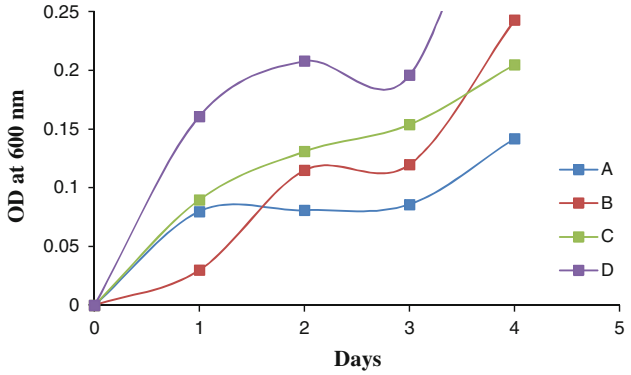


Fig. 2 Substrate utilization patterns of consortia trials

polar water molecules are repelled from the hydrophobic surface. In contrast, if the water droplet contains surfactant, the force or interfacial tension between the water drop and the hydrophobic surface is reduced, which results in the spreading of the water drop over the hydrophobic surface.

Thin-layer chromatography

The chromatogram on TLC showed the presence of 4 spots on the tested samples whereas the control with oil gave only 2 spots (Fig. 3). The control with oil gave two spots at the Rf value of 0.69 and 0.93. The samples from the tested culture gave 4 spots on TLC with the Rf value of 0.18, 0.36, 0.69 and 0.93, respectively. The presence of two extra spots at Rf of 0.18 and 0.36 indicates the degradation of oil by the tested microbial consortia.

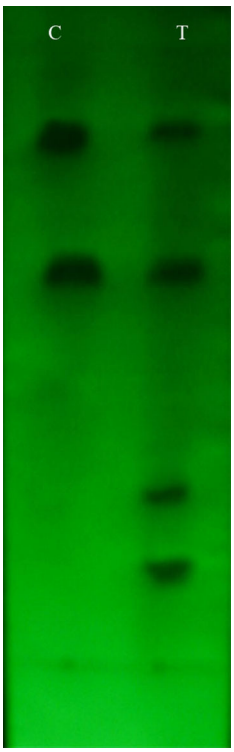


Fig. 3 Thin layer chromatography of degraded oil. *C* control with two spots, *T* test with four spots

Column chromatography

The chromatogram on column packed with silica and activated alumina gave significant fractions of degraded oils. Compared to chromatogram of control which had only two peaks (Fig. 4a), the chromatogram of test sample had four peaks (Fig. 4b). The increase in number of peaks in test sample depicts that oil used as substrate has been fragmented into smaller compounds. This also greatly corresponds with chromatogram of TLC. Therefore, organisms used in consortia are actually capable of degrading the oil substrates into smaller compounds and then utilize them for their growth. Prokaryotes convert aromatic hydrocarbons by an initial dioxygenase attack, to *trans*-dihydrodiols that are further oxidized to dihydroxy products, e.g., catechol in the case of benzene (Atlas and Bartha 1998).

Bioremediation studies

Results based on gravimetric analysis of oil of the treated and untreated soils are represented in Table 5. All the soils behaved differently during treatment processes with

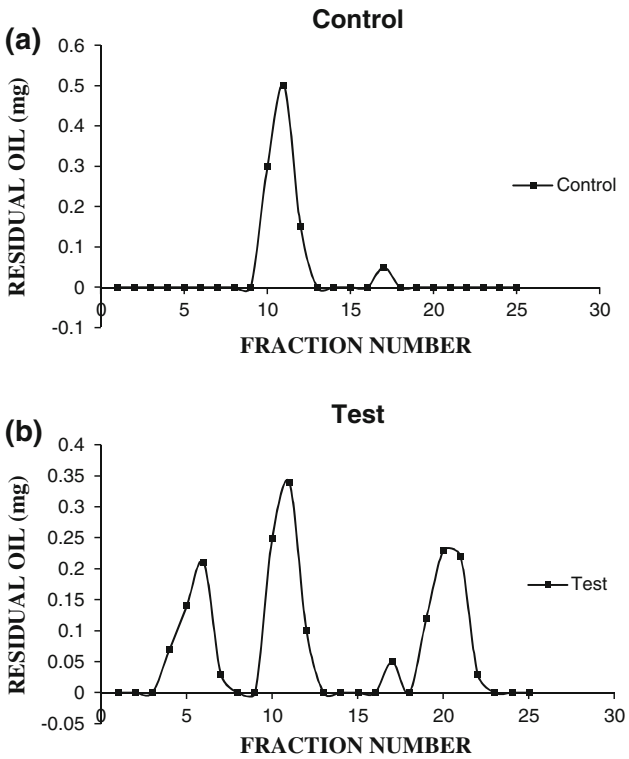


Fig. 4 **a** Chromatogram of control oil. **b** Chromatogram of fragmented oil by consortial microbes

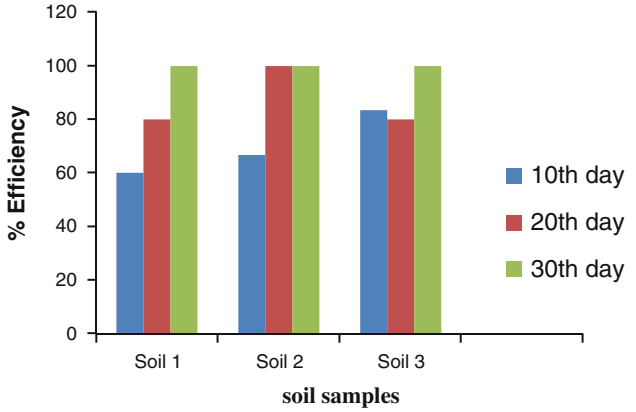


Fig. 5 Efficiency of consortia in treating oil-contaminated soils

respect to residual oil content. The microbial consortia contained in the treatment group reduced the residual oil levels in the soil effectively. The group treated with microbial consortium reduced the residual oil level to zero on the 30th day of analysis in all the three tested soil samples (Table 5 and 6) (Fig. 5). Studies have shown that bacterial degradation was altered greatly by the physical and chemical features of the heterogeneous microbial environment (Tang et al. 2005).

Biodegradation kinetics

Microbial growth on pollutant mixture is an important aspect of bioremediation treatment. For both in situ and ex situ operations such strategies are developed. When individual microbial species are considered, simple competition for the growth substrate is the only interaction included (Greene et al. 2000). Here, our results are presented using *Pseudomonas* sp. and *Bacillus* sp., their possible consortia growing individually on oil-contaminated soils and mathematical models are compared to describe these results.

The general formula of the first-order kinetic that can describe the rate of total oil reduction is.

$$\frac{dC}{dT} = -kt \tag{1}$$

where t, time (day), C remaining oil concentration (mg/g) at any time, k first-order kinetic constant (1/day).

Equation 1 assumes that the microbial concentration remains constant over the entire experimentation time. Therefore, the effect of microbial concentration on the kinetics constant is neglected.

The integration of Eq. 1 leads to the known formula of the first-order kinetics.

Table 5 Bioremediation of contaminated soils

Soils	Control (oil in mg/g of soil)			Sample (oil in mg/g of soil)		
	10th day	20th day	30th day	10th day	20th day	30th day
1	0.05	0.05	0.05	0.02	0.01	0
2	0.03	0.03	0.03	0.01	0	0
3	0.06	0.05	0.05	0.01	0.01	0

Table 6 Efficiency of consortial treatment

Soils	Sample (efficiency %)		
	10th day	20th day	30th day
1	60	80	100
2	66.67	100	100
3	83.34	80	100

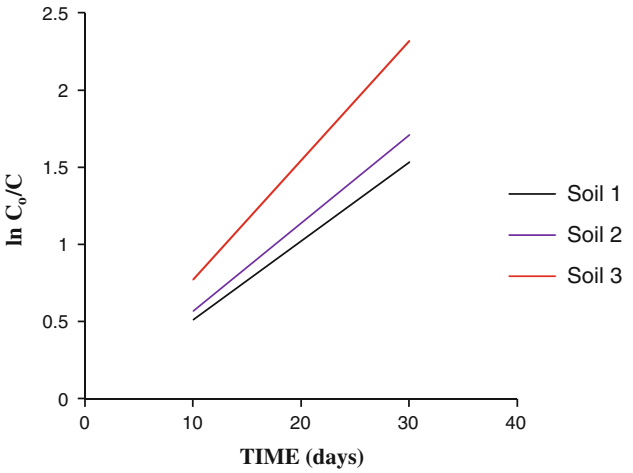


Fig. 6 Linearization of experimental data in soils

$$C = C_0 e^{-kt} \tag{2}$$

where C_0 is the initial oil concentration (mg/g of soil).

$$\ln \frac{C_0}{C} = kt \tag{3}$$

In order to experimentally calculate the kinetic constant k, Eq. 2 is linearized.

The first-order kinetics is said to be valid if a linear relationship is achieved upon plotting the logarithmic plot of Eq. 3 versus time. Most of the compounds obeyed first-order kinetics for hydrocarbon removal (Bonaventura and Johnson 1996). The slope of the line

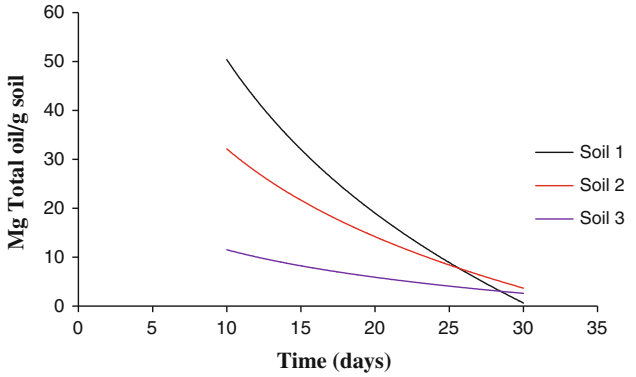


Fig. 7 First-order kinetic curve in soils

Table 7 First-order kinetic constants for bioremediation process by consortia

Trials	Mg total oil/g soil	Kinetic constant k (day^{-1})	R^2
Soil 1	0.5	0.051	0.972
Soil 2	0.3	0.057	0.963
Soil 3	0.6	0.077	0.991

Table 8 Phytotoxicity parameters in treated and untreated soils

S. no	Soil	Germination index (%)	Average growth index (cm)	Sustenance index (%)
1	Plain soil (control)	60	4	95.66
2	Untreated refinery soil	40	0.5	23.5
3	Treated refinery soil	50	2	73.33

represents the first-order kinetic constant k. The linearization results of the experimental data are graphically presented in Figs. 6 and 7. The kinetic constants for all runs calculated from the slopes of the linearization are summarized in Table 7.

The results in Table 7 show that the first-order kinetic constants for the different soils at different initial oil content ranging between 0.051 and 0.077/day. These results agree in a great extent with the results achieved in previous studies (Hutchins et. al. 1991; Bock et al. 1994; Boonchan et al. 2000).

The validation of the first-order kinetics was tested using the experimental results that were also reported in previous study (Reardon et al. 2002). The model validation results are graphically presented in Figs. 6 and 7. These figures show that the first-order kinetic can be used with relatively considerable accuracy in order to describe the bioremediation process of hydrocarbon contaminated soil.

The first-order rate kinetic constants found in this work are similar to that found in different previous studies. (Hutchins et al. 1991) reported that first-order rate constant ranged from 0.016 to a high of 0.38/day depending on compounds of hydrocarbon. (Hwang et al. 2001) found that the first-order kinetic rate constant of diesel oil was 0.099/day. Previous studies showed that the first-order kinetic constant at 38 °C was 0.013/day (Antizar-Ladislao et al. 2005).

Plantation studies

As an experiment, fenugreek seeds were planted separately in treated and untreated soils. After few days, it was observed that, the growth characteristics of plants were better in treated soils compared to untreated soils. Also, it was observed that the bioremediation process detoxified the phytotoxic property of contaminated soils and restored its lost fertility (Table 8). This study corresponds with (Millioli et al. 2009) who observed that the increase in the Biosurfactant rhamnolipid concentration increased the germination index (GI) and decreased the acute toxicity (EC50 values), of *Lactuca sativa*. Nevertheless, at the lowest concentration (1 mg g^{-1}), an increase in the GI was observed, reaching a value above the natural soil, which suggests that low concentrations may increase the availability of some nutrients improving soil fertility.

Conclusion

It was observed that even though some microbes utilized one or two substrates individually were unable to utilize those substrates in combination. Therefore, the capability of consortia in utilizing all forms of substrates efficiently was assessed and used directly in bioremediation studies. Set 'D' consortia were observed to be very effective in treating all kinds of experimental soils with different residual oil content. The efficiency was recorded as almost 100 % in about 30 days. Additionally, the biodegradation potential of consortia was analyzed through bioremediation kinetics which resulted in the first-order kinetic constants for different soils with different initial oil content (range 0.051–0.077/day). Detoxification of soil phytotoxicity

because of pollutant oil was done and the results observed showed an increase of 25, 300 and 212 % in germination index, average growth index and sustenance index, respectively, of *Trigonella foenum-graecum* in treated soils compared to the untreated ones. Therefore, it is recommended that consortia 'D' could be effectively used for bioremediation of oil spills in soils and conventionally oil-contaminated soils. The soil fertility could also be restored for future agricultural practices.

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References

Abdulsalam S, Bugaje IM, Adefila SS, Ibrahim S (2011) Comparison of biostimulation and bioaugmentation for remediation of soil contaminated with spent motor oil. Int J Environ Sci Tech 8(1):187–194

Alexander M (1999) Biodegradation and Bioremediation, 2nd edn. Academic Press, San Diego, p 350

Antizar-Ladislao B, Lopez-Real J, Beck AJ (2005) Laboratory studies of the remediation of polycyclic aromatic hydrocarbon contaminated soil by in-vessel composting. Waste Manage 25(3):281–289

Atlas RM, Bartha R (1998) Fundamentals and Applications. Microbial Ecol, 4th edn. Benjamin/Cummings Publishing Company, Inc, California, pp 523–530

Banks MK, Schultz KE (2005) Comparison of plants for germination toxicity test in petroleum contaminated soil. Water Air Soil Poll 167(1-4):211–219

Bock M, Kampfer K, Dott W (1994) Isolation and characterization of heterotrophic aerobic bacteria from oil storage caverns in northern Germany. Appl Microbial Biotechnol 42(1):463–468

Boehm PD, Douglas GS, Brown JS (1995) Advanced chemical fingerprinting for oil spill identifications and natural resource damage assessments. In: Proceedings of the 1995 International oil spill conference, American Petroleum Institute, Long Beach, California, pp 967–969

Bonaventura C, Johnson FJVI (1996) Healthy environment for healthy people: bioremediation now and tomorrow. Environ Health Persp 105(1):5–21

Boonchan S, Britz ML, Stanley GA (2000) Degradation and mineralization of high-molecular weight polycyclic aromatic hydrocarbons by defined fungal-bacterial co cultures. Appl Environ Microbiol 66(3):1007–1019

Brito EMS, Guyoneaud R, Goñi-Urriza M, Ranchou-Peyruse A, Verbaere A, Crapez MA, Wasserman JCA, Duran R (2006) Characterization of hydrocarbonoblastic bacterial communities from mangrove sediments in Guanabara Bay Brazil. Res Microbiol 157(8):752–762

Chaillana F, Flècheb A, Burya E, Phantavonga Y, Saliot A, Oudot J (2004) Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms. Res Microb 155(7):587–595

Chang R (1998) Chemistry, 6th edn. McGraw–Hill Companies, Inc, New York, pp 962–963

Fletcher J (1991) A brief overview of plant toxicity testing. In: Plants for toxicity Assessment, vol 2, ASTMSTP, Philadelphia, PA

Ghazali MF, Zaliha NR, Salleh B, Basri M (2004) Biodegradation of hydrocarbons in soil by microbial consortium. Inter Biodeter Biodegrad 54(1):61–67

Greene EA, Kay JG, Jaber K, Stehmeier G, Voordouw G (2000) Composition of soil microbial communities enriched on a mixture of aromatic hydrocarbons. Appl Environ Microbiol 66(12):5282–5289

Holliger C, Gaspard S, Glod G, Heijman C, Schumacher W, Schwarzenbach RP, Vazquez F, Gaspard S (1997) Contaminated environments in the subsurface and bioremediation: organic contaminants. FEMS Microbiol Rev 20(3–4):517–523

Hutchins SR, Sewell GW, Kovacs DA, Smith GA (1991) Biodegradation of aromatic hydrocarbons by aquifer microorganisms under denitrifying conditions. Environ Sci Technol 25(1):68–76

Hwang E, Namkoong W, Park J (2001) Recycling of remediated soil for effective composting of diesel-contaminated soil. Compost Sci Util 9(2):143–149

Lliros M, Munill X, Sole A, Martinez-Alonso, Diestra E, Esteve I (2003) Analysis of cyanobacteria biodiversity in pristine and polluted microbia mats in microcosmos by confocal laser scanning microscopy (CLSM). In: Méndez-Vilas A (ed) Science, Technology and Education of Microscopy: An Overview, vol 1. Formatex Research Center, Badajoz, Spain, pp 483–489

Mandri T, Lin J (2007) Isolation and characterization of engine oil degrading indigenous microorganisms in Kwazulu-Natal South Africa. Afr J Biotechnol 6(1):23–27

Marquez-Rocha FJ, Hernandez-Rodriguez V, Lamela MT (2001) Biodegradation of engine and diesel oil in soil by a microbial consortium. Water Air Soil Poll 128(1):313–320

Millioli VS, Servulo E-LC, Sobral LGS, de Carvalho DD (2009) Bioremediation of crude oil-bearing soil: evaluating the effect of rhamnolipid addition to soil toxicity and to crude oil biodegradation efficiency. Glob NEST J 11(2):181–188

Mittal A, Singh P (2009) Isolation of hydrocarbon degrading bacteria from soils contaminated with crude oil spills. Indian J Exp Biol 47(9):760–765

Nester EW, Anderson DG, Evans Roberts C Jr, Pearsall NN, Nester MT (2001) Microbiology: a human perspective, 3rd edn. McGraw-Hill, New York, p 120

Radwan SS, Sorkhoh NA (1993) Lipids of n-alkanes utilizing microorganisms and their application potential. Adv Appl Microbiol 39(1):29–90

Reardon KF, Mosteller DC, Rogers JB, DuTeau NM, Kee-Hong K (2002) Biodegradation kinetic of aromatic hydrocarbon mixtures by pure and mixed bacterial cultures. Environ Health Perspec 110(1):1005–1012

Tang Y, Qi JL, Krieger-Brockett B (2005) Evaluating factors that influence microbial phenanthrene biodegradation rates by

regression with categorical variables. Chemosphere 59(5):729–741

Thouand G, Bauda P, Oudot J, Kirsch G, Sutton C, Vidalie JF (1999) Laboratory evaluation of crude oil biodegradation with commercial or natural microbial inocula. Can J Microb 45(2):106–115

Tugrul T, Cansunar E (2005) Detecting surfactant-producing microorganisms by the drop-collapse test. World J Microbiol Biotechnol 21(1):45–49

Vidali M (2001) Bioremediation. An overview. Pure Appl Chem 73(7):1163–1172