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RESEARCH ARTICLE

Bioremediation and reclamation of soil contaminated with petroleum oil hydrocarbons by exogenously seeded bacterial consortium: a pilot-scale study

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Abstract
Purpose Spillage of petroleum hydrocarbons causes significant environmental pollution. Bioremediation is an effective process to remediate petroleum oil contaminant from the ecosystem. The aim of the present study was to reclaim a petroleum oil-contaminated soil which was unsuitable for the cultivation of crop plants by using petroleum oil hydrocarbon-degrading microbial consortium.
Materials and methods Bacterial consortium consisting of *Bacillus subtilis* DM-04 and *Pseudomonas aeruginosa* M and NM strains were seeded to 20% (*v*/*w*) petroleum oil-contaminated soil, and bioremediation experiment was carried out for 180 days under laboratory condition. The kinetics of hydrocarbon degradation was analyzed using biochemical and gas chromatographic (GC) techniques. The ecotoxicity of the elutriates obtained from petroleum oil-contaminated soil before and post-treatment with microbial consortium was tested on germination and growth of Bengal gram (*Cicer aretinum*) and green gram (*Phaseolus mungo*) seeds.
Results Bacterial consortium showed a significant reduction in total petroleum hydrocarbon level in contaminated soil (76% degradation) as compared to the control soil (3.6% degradation) 180 days post-inoculation. The GC analysis confirmed that bacterial consortium was more effective in degrading the alkane fraction compared to aromatic fraction of crude petroleum oil hydrocarbons in soil. The nitrogen, sulfur, and oxygen compounds fraction was least degraded. The reclaimed soil supported the germination and growth of crop plants (*C. aretinum* and *P. mungo).* In contrast, seeds could not be germinated in petroleum oil-contaminated soil.
Conclusions The present study reinforces the application of bacterial consortium rather than individual bacterium for the effective bioremediation and reclamation of soil contaminated with petroleum oil.

Keywords Bioremediation · Crop plants · Petroleum oil hydrocarbon degradation · Soil reclamation · *Bacillus subtilis* · *Pseudomonas aeruginosa*

1 Background, aim, and scope

Crude petroleum oil and its derivatives released in the environment either accidentally or deliberately pose a problem of increasing magnitude throughout the world (Okoh and Trejo-Hernandez 2006). Furthermore, this problem is more aggravated because of the expensive disposal methods (Das and Mukherjee 2007a; Rahman et al. 2003). It should be noted that site remediation technology must be simple and cost-effective for both industrialized as well as for developing countries to be able to sort out and resolve this menace.

Among the various strategies adopted to clean crude petroleum oil-contaminated soil, bioremediation is recognized as one of the effective and inexpensive technologies (Das and Mukherjee 2007a). This approach involves supplying hydrocarbon-degrading efficient microorganisms to degrade target compounds, along with appropriate nutrients, to the subsurface (petroleum oil-contaminated sites). Bioremediation of complex hydrocarbons usually requires the cooperation of more than a single species because the individual microorganism can metabolize only a limited range of hydrocarbon substrates. Therefore, assemblages

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of mixed populations with overall broad enzymatic capabilities are required to bring the rate and extent of petroleum hydrocarbon degradation much faster (Wiesel et al. 1993). Some members of the microbial community might be able to secrete important degradative enzymes, growth factors, whereas others may produce biosurfactants leading to the enhanced solubilization of hydrophobic hydrocarbons for their better utilization by microbes (Ghazali et al. 2004; Mukherjee and Das 2005).

A perusal of the literature shows that during the last decade, several reports on the microbial degradation of petroleum oil hydrocarbons in soil were published; however, limited studies have been attempted to investigate the ecotoxicity and reclamation of bioremediated soil. Since the major objective of the bioremediation of petroleum oil-contaminated soil should be to reclaim the soil, enabling it to support the growth of crop plants, therefore, the present study was initiated with an aim to reclaim 20% (*v*/*w*) petroleum oil-contaminated soil by using a consortium of bacteria consisting of *Bacillus subtilis* DM-04 and *Pseudomonas aeruginosa* M and NM strains. It is worthy to mention that these bacteria originally isolated from a petroleum oil-contaminated soil were shown to be efficient degraders of petroleum oil components both in liquid culture as well as in soil (Mukherjee and Das 2005; Das and Mukherjee 2007a). Furthermore, our study has demonstrated that bioremediated soil can support the germination and growth of crop plants, thus vouching for the efficiency of this method.

2 Materials and methods

2.1 Biodegradation of petroleum oil hydrocarbons and its fractions in soil by bacterial consortium

Isolation and taxonomic identification of pure culture of bacteria used in the present study, viz. *B. subtillis* DM-04, *P. aeruginosa* M, and *P. aeruginosa* NM strains, have been described previously (Das and Mukherjee 2005; Mukherjee and Das 2005). Briefly, 5 g of hydrocarbon-contaminated soil was transferred to a 500-ml Erlenmeyer flask containing 100 ml of modified M9 medium containing 2% (*v*/*v*) hexadecane and incubated on a rotary shaker at 45°C and 200 rpm. From there, 0.1 ml of culture was plated on nutrient agar plates and incubated at 45°C for 24 h, and morphologically different bacteria were selected based on their biosurfactant production and hydrocarbon degradation property. These biosurfactant-producing and petroleum oil hydrocarbon-degrading bacterial strains (*B. subtillis* DM-04, *P. aeruginosa* M, and *P. aeruginosa* NM) were maintained on nutrient agar slants and subcultured every 2 weeks and also preserved in 20% (*v*/*v*) glycerol and stored at −80°C.

For the laboratory-scale bioremediation experiment, a bacterial consortium was formulated by mixing equal proportions of pure cultures of *B. subtillis* DM-04, *P. aeruginosa* M, and *P. aeruginosa* NM strains (1×10^9 cfu l^{-1}). The biodegradation efficiency of the bacterial consortium on the crude petroleum oil-contaminated soil (20%, *v*/*w*) was assessed over a period of 180 days of post-inoculation (April–September) under laboratory condition (Das and Mukherjee 2007a) with the following modifications. Briefly, 5.0 kg of soil contaminated with 180 g kg^{-1} crude petroleum oil [equivalent to 20% (*v*/*w*) contamination] was layered in rectangular trays of 30×40×10-cm (length × breadth × height) sizes. The depth of the soil in each tray was maintained at 5.0 cm, and the water holding capacity, as well as the moisture content of the soil, was determined. Prior to initiating the biodegradation experiment, each tray was treated with a 50 ml aqueous solution of biosurfactant at a concentration of 1.0 g l^{-1}, which was prepared by mixing the biosurfactant obtained from the respective bacterial strain in equal proportion (1:1:1, *w*/*w*). The lipopeptide biosurfactant production from *B. subtilis* strain DM-04 was carried out in a submerged fermentation using potato peel as a substrate (Das and Mukherjee 2007b), whereas M9 medium adjusted to pH 7.0 was used for biosurfactant production by *P. aeruginosa* M and NM strains at 45°C (Das and Mukherjee 2005).The soil samples were biostimulated by adding NH_4NO_3 and KH_2PO_4/Na_2HPO_4 as nitrogen and phosphorus sources, respectively, to give an initial C/N/P ratio of approximately 100:10:1 (Vezquez et al. 2009). The soil was then left for 72 h at ambient temperature and at 75–85% humidity followed by inoculation with 250 ml of bacterial consortia (see above) at a density of 1×10^9 cfu l^{-1}. The soil in the tray was thoroughly tilled at a regular interval of 15 days, and the liquid mineral medium was sprayed for maintenance of its moisture and nutrient level. One hundred milliliters of glucose solution (250 mg l^{-1}) was supplemented to each tray at an interval of 30 days and continued for up to 120 days. It is to be noted that the supplementation of glucose as a co-carbon source enhanced the rate of biodegradation of PAH by bacterial strains used in this study (Das and Mukherjee 2007a). To assure the reproducibility, each experiment was run in triplicate. A control was run in parallel where the soil was treated with un-inoculated medium.

The residual total petroleum hydrocarbons (TPH) as well its various fractions remaining in soil in the post-bioremediation experiment was analyzed (see below) and compared with the control (untreated soil). Total microbial count (viable cell count) of the soil on the onset and after the end of the experiment was determined with standard dilution plating technique using sterile nutrient agar (Difco) growth medium. Survival of *B. subtilis* DM-04, *P. aeruginosa* M, and NM strains in

the petroleum oil hydrocarbon-rich soil was determined as described previously (Das and Mukherjee 2007a).

2.2 Analysis of soil

Moisture, pH, and water holding capacity of the soil were determined as described previously (Das and Mukherjee 2007a). Briefly, for the determination of pH, 10 g of air-dried soil sample was suspended in 20 ml of distilled water, mixed well, and then allowed to stand for 1 h at room temperature. The pH of the water post-sedimentation of soil was measured using a pH meter (Cyberscan 510, Merck). Total nitrogen content of soil was determined by the Kjeldahl method (Kjeldahl 1883). The amount of potassium available in the soil sample was determined by first extracting it with ammonium acetate and than quantified using a flame photometer. The total phosphorus content of the soil was determined by percholic acid digestion and sodium carbonate fusion method (Mattingly 1970). For determining the moisture content, 100 g of petroleum oil - contaminated soil sample was taken in a pre-weighed breaker, dried overnight at 60°C, and then kept in a desiccator. The sample was then ground in a mortar, sieved using 36-mesh size sieve, re-dried, and then weighed. The loss in weight was calculated as the moisture content of the soil (Das and Mukherjee 2007a).

2.3 Estimation of total petroleum hydrocarbon and its fractions in soil

TPH was extracted from 10 g of soil and separated into aromatic, aliphatic, asphaltene, and nitrogen, sulfur, and oxygen compounds (NSO) fractions as described previously (Das and Mukherjee 2007a). Briefly, TPH from 10.0-g soil was consecutively extracted with 100 ml of hexane, methylene chloride (CH_2Cl_2), and chloroform. All three extracts were pooled and dried in a fume hood at room temperature by evaporation of solvents under a gentle nitrogen stream over Na_2SO_4 and concentrated using a rotary evaporator to a final volume of 3.0 ml. After solvent evaporation, the amount of residual TPH was determined gravimetrically. Residual TPH was then fractionated into alkane, aromatic, asphaltene, and NSO-containing fractions on a silica gel column (Das and Mukherjee 2007a). The alkane and aromatic fractions were analyzed using gas chromatography–mass spectroscopy (Varian CP-3800, GC) coupled with a CP-Sil 8 CB, low bleed 30 m×0.25 mm× 0.25 m column (for GC analysis), and CP-Sil 5 CB low bleed/MS column. The column temperature was kept at 80–240°C for 30 min with 5°C min^{-1} increment and hold at 240° C for 30 min. The injector temperature was 240°C and the injector was in the splitless mode. The detector temperature was programmed at 300°C. The mass spectrometric data were acquired in electron ionization mode (70 eV). The individual components in the alkane and aromatic fractions were determined by matching the retention time with the authentic standards and/or MS library search (Das and Mukherjee 2007a).

2.4 Soil reclamation and ecotoxicity study

The ecotoxicity of the elutriates obtained from petroleum oil-contaminated soil before and post-treatment with microbial consortium at different time intervals was tested on germination of Bengal gram (*Cicer aretinum*) and green gram (*Phaseolus mungo*) seeds (Petukhov et al. 2000). For soil reclamation study, 2.0 kg each of the following soil sample was taken in an individual earthen pot: normal (uncontaminated) soil (C); soil contaminated with 20% (*v*/*w*) crude petroleum oil (S_0); and crude petroleum oil-contaminated soil (20%, *v*/*w*) treated with bacterial consortium for 30 days (S_1), 90 days (S_2), and 180 days (S_3). Similar to the treatment of soil under bioremediation experiment, prior to starting the experiment, normal soil was also mixed with NH_4NO_3 and KH_2PO_4/Na_2HPO_4 as nitrogen and phosphorus sources, respectively, to give an initial C/N/P ratio of approximately 100:10:1. Each soil was mixed with organic manure (dried cow dung) at a dose of 125 g kg^{-1} of soil, and pots were maintained for 28 days at 28±2°C. Overnight pre-soaked seeds of Bengal gram and green gram were allowed to germinate in each soil, and germination time and percent seed germination in each soil were determined. Plant growth was monitored at an interval of 7 days and continued for a period of 1 month. Each experiment was performed in triplicate to assure the reproducibility.

2.5 Statistical analysis

Statistical analysis was done by determining the level of significance (5% level) between the two sets of data by Student's *t* distribution (*p* value) analysis. Both the confidence coefficient and degree of freedom were taken into account when using the table of *t* distribution.

3 Results

3.1 Biodegradation of TPH and various fractions of crude petroleum oil in soil

There are very few reports available on the in situ biodegradation of crude petroleum oil hydrocarbons in soil when the contamination level was >10%. Soil sample contaminated with 20% crude petroleum oil selected for the bioremediation experiment was loamy and dark brown in color. The percent moisture content of the soil was

determined as 18±2 (n=3). The pH of the soil was 6.5–6.8 before the biodegradation experiment (time 0) and did not change significantly at the end of the experiment. The water holding capacity of the soil increased from 45% to 70% at the end of the experiment. The level of TPH contamination in soil was detected as 179.5 g kg^{-1} of soil. The alkane fraction, which is the largest constituent of TPH, was detected as 56% of total TPH followed by aromatic fraction (20%), asphaltene fraction (16%), and then NSO (5%) fraction (Table 1). Indigenous bacterial population (viable cell count) of petroleum oil-contaminated soil was determined as 1×10^7 cfu kg^{-1} of soil.

The biodegradation study showed that the TPH level of contaminated soil was reduced from 179.5 to 43 gkg^{-1} (76% degradation) 180 days post-treatment with bacterial consortium (Table 1). The in situ degradation of TPH, alkane, aromatic, NSO, and asphaltene fractions by indigenous microbes present in petroleum oil-contaminated soil (control soil) was insignificant ($p>0.1$) compared to the biodegradation of the same fractions by applied bacterial consortium (Table 1). The rate of biodegradation of n-alkanes by bacterial consortium was found to be significantly higher ($p<0.05$) compared to biodegradation of aromatic or NSO fractions. The plate count indicated expressive growth of exogenously added bacteria in petroleum oil-contaminated soil, and their cell densities ranged from $1\times10^9-2\times10^{15}$ $cfukg^{-1}$ as compared to control soil (1×10^7–1×10^9 cfu kg^{-1} soil)

3.2 Analysis of aliphatic and aromatic fractions by GC

The GC analysis confirmed that bacterial consortium was more effective in degrading the alkane fraction compared to the aromatic fraction of crude petroleum oil hydrocarbons in soil (Fig. 1a–d). The percent degradation of alkanes was observed as C_8–C_{11} (75%), C_{12}–C_{21} (98%), and C_{22}–C_{40} (92%), and this degradation was higher than the previous report on medium-chain alkane (C_{15}–C_{22}) degradation by consortium 2 (Ghazali et al. 2004).

Benzene, toluene, and xylene (BTX) degradation in soil showed that added bacterial consortium preferentially degraded the benzene (98%) followed by toluene (96%), whereas xylene (76%) was least degraded in soil after 180 days of treatment with bacterial consortium (Fig. 2a–d).

3.3 Reclamation and ecotoxicity test of bioremediated soil

Perusal of the literature shows that limited attempt has been made to investigate the ecotoxicity and reclamation of bioremediated soil. It was observed that both Bengal gram and green gram seeds failed to germinate in crude petroleum oil-contaminated as well as 30-day post-bioremediation soils, whereas 30–33% of the seeds could be germinated in 90-day post-bioremediation soil (data not shown). Nevertheless, the percentage of seed germination (72–74%) in normal (without contamination) and 180-day bioremediated soils was essentially the same ($p>0.05$). The higher germination percentage of both types of seed in S_3 soil compared to S_1 and S_2 soils might be related to the lower toxicity level and higher degradation and utilization of petroleum hydrocarbons by bacterial consortium.

The bioremediation efficiency of the seeded bacterial consortium was further assessed on the basis of growth of plants on bioremediated soil. Bengal gram and green gram exhibited different patterns of growth in C, S_1, S_2, and S_3 soils (Table 2). In S_0 and S_1 soils, the seeds failed to germinate. Interestingly, plant growth rate was found to be higher in 180-day bioremediated soil (S_3) compared to the normal (C) soil, indicating that the residual compounds left

Table 1 Biodegradation of various fractions of crude petroleum oil hydrocarbons in soil by bacterial consortium

Contaminants	Level of contamination in soil (gkg^{-1} of soil)				
	Before the experiment (day0)	After the experiment			
		90 Days post-treatment		180 Days post-treatment	
		None (control)	Bacterial consortium	None (control)	Bacterial consortium
TPH	179.5±9.2	178±8.1	106±8.5	173±4.5	43±3.3
Alkane fraction	103±7.1	98±8.3	64±5.5	90±8.2	6±1.1
Aromatic fraction	35±3.3	34±2.9	16±2.3	31±3.2	9±1.0
NSO and asphaltene fraction	36±3.2	35±2.1	27±4.1	29±3.0	22±2.2

Each value represents mean ±S D of results obtained from three individual experimental trays

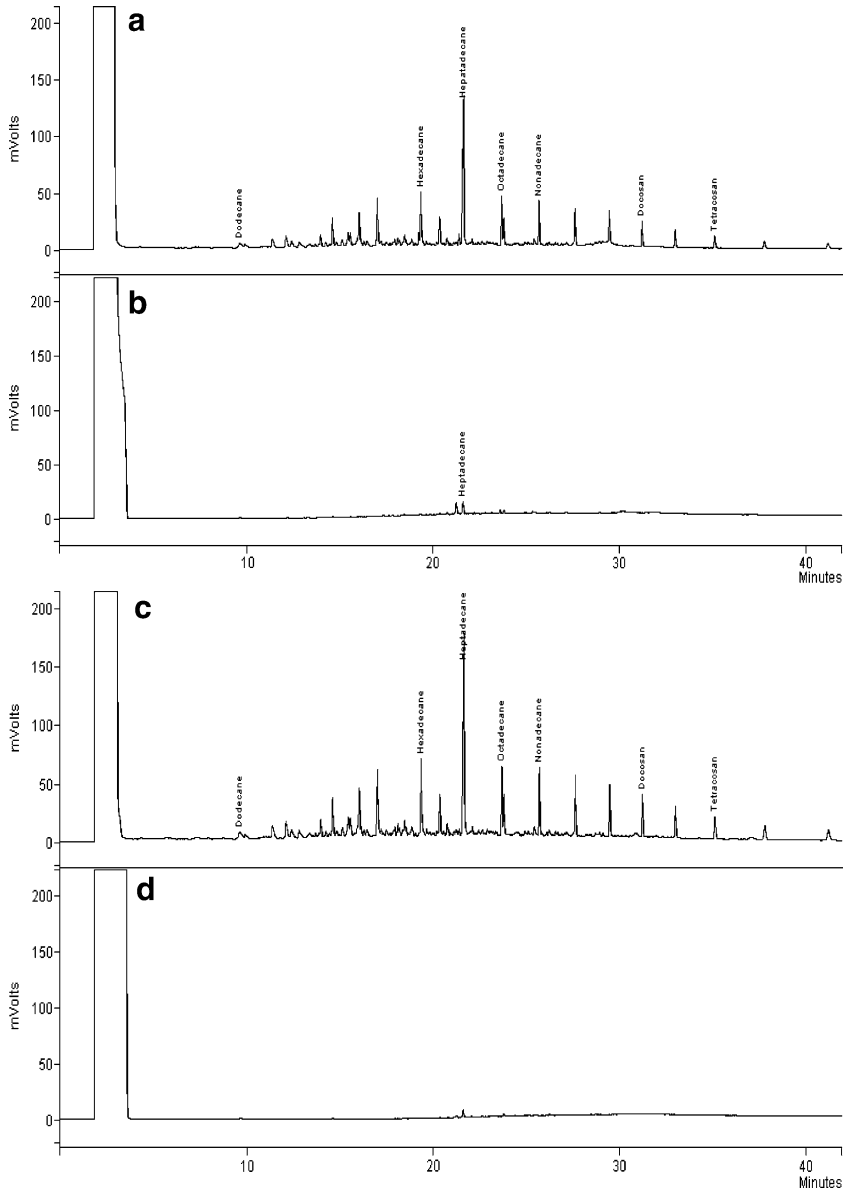

Fig. 1 **a**–**d** GC fingerprinting of the aliphatic fraction of crude petroleum oil from soil seeded with *P. aeruginosa* M, NM, and *B. subtilis* DM-04 consortium: **a** At day 0 (beginning of bioremediation experiment); **b** After 120 days post-treatment with bacterial consortium; **c** After 180 days without exogenously seeded bacteria (control); **d** After 180 days post-treatment with bacterial consortium

out from the contaminating crude petroleum oil components were conducive to the growth and development of plants.

3.4 Nutrients and pH status of bioremediated soil

Data generated on the determination of pH and nutrient status of bioremediated soil are presented in Table 3. In the case of bioremediated soil, the level of N (pot, 2.9-mg cm^{-2} pot) and K (pot, 4.5 mg cm^{-2}) was found to be higher than the normal soil (pot, 2.5 mg cm^{-2}; K pot, 3.3 mg cm^{-2}). Phosphorous level was found to be more or less similar to that of normal soil. There was no difference in pH of the bioremediated and normal soils.

4 Discussion

4.1 Biodegradation of TPH and various fractions of crude petroleum oil in soil

The rate of biodegradation of TPH by the seeded bacterial consortium is the key deciding factor for the successful reclamation of soil for the cultivation of crop plants. The in situ biodegradation of TPH and its fractions by bacterial consortium was found to be higher than the previously reported values for TPH degradation in soil by individual isolate as well as by other bacterial consortia (Das and Mukherjee 2007a; Ghazali et al. 2004; Obayori et al. 2009). It has been suggested that synergistic interaction among the

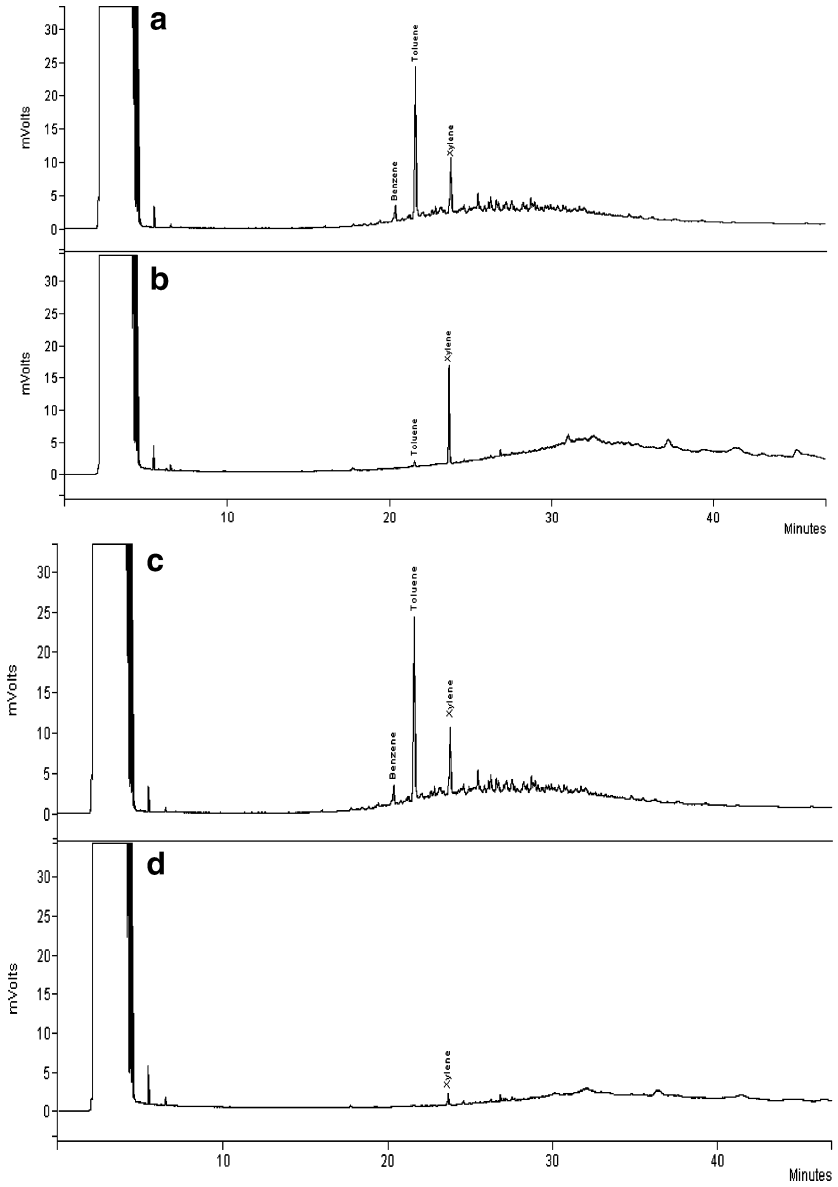

Fig. 2 **a**–**d** GC fingerprinting of the aromatic fraction of crude petroleum oil from soil seeded with *P. aeruginosa* M, NM, and *B. subtilis* DM-04 consortium: **a** at day 0 (beginning of bioremediation experiment); **b** After 120 days post-treatment with bacterial consortium; **c** After 180 days without exogenously seeded bacteria (control); **d** After 180 days post-treatment with bacterial consortium

Table 2 Germination and growth performance of green gram and Bengal gram in control and 180-day bioremediation soils

Soil types	Bengal gram (*Cicer aretinum*)		Green gram (*Phaseolus mungo*)	
	Shoot height (cm)	No of branches	Shoot height (cm)	No. of branches
C	15.3±0.2	3.4±0.3	10.2±0.6	2.5±0.2
S_0	NG	NB	NG	NB
S_1	NG	NB	NG	NB
S_2	9.4±0.4	2.5±0.2	5.5±0.3	2.2±0.1
S_3	19.2±0.5	5.3±0.2	17.3±0.7	3.4±0.2

Growth of plants was measured 30 days post-seeding. Each soil was seeded with five seeds, and values are mean ± SD of results from the three individual pots

NG no germination, *NB* no branching

members of bacteria in a community is one of the essential factors for achieving successful bioremediation of soil (Ghazali et al. 2004; Kim et al. 2005). The least degradation of NSO compounds might be due to their lower bioavail-

Table 3 Nutrient (NPK) and pH status of the bioremediated and normal soils used for the germination and evaluation of plant growth

Soil types	pH	N ($mgcm^{-2}$)	P_2O_5 ($mgcm^{-2}$)	K_2O ($mgcm^{-2}$)
Normal	5.5±0.2	2.5±0.1	0.2±0.1	3.3±0.2
Bioremediated	5.6±0.1	2.9±0.2	0.3±0.1	4.5±0.1

Values are mean ± SD of results from the three individual pots

ability in soil microcosm for microbial attack (Das and Mukherjee 2007a; Dyreborg et al. 1989).

4.2 Analysis of aliphatic and aromatic fractions by gas chromatography

Crude petroleum oil is a complex mixture of hydrophobic components like *n*-alkane, aromatics, resins, and asphaltines, and microorganisms are known to attack a specific component in comparison to other components of petroleum oil. GC-FID analysis revealed that degradation of TPH was higher than the previous report on medium-chain alkane degradation by consortium 2 (Ghazali et al. 2004). It may therefore be inferred that *n*-alkanes, particularly with a hydrocarbon chain length in between C_{12} and C_{21}, are the most suitable substrates for microbial attack owing to their low hydrophobicity (Ghazali et al. 2004). Furthermore, bioavailability is the key deciding factor for the biodegradation of any component of crude petroleum oil. Therefore, it may be presumed that the lower degradation of short-chain alkanes (C_8–C_{11}) is related to their less availability due to the easy penetrability in soil (Hornick et al. 1983) as well as their toxicity to bacterial cells (Wang et al. 2002).

The BTX degradation potential of the formulated bacterial consortium was significantly higher as compared to the rate of biodegradation of these compounds by individual isolate of the same consortium and also better than some previously reported values on the degradation of BTX with individual isolates (Jean et al. 2008; Wang et al. 2008). This result reinforces the application of bacterial consortium rather than individual member of the same consortium for effective bioremediation. Studies on metabolic pathways for BTX removal under aerobic condition have revealed that benzene is degraded to catechol while toluene is degraded via several separate pathways to 3-ethylcatechol (Johnson et al. 2003). Xylene is metabolized to mono-methylated catechol. Therefore, it is quite obvious that bacteria in a combination would be able to utilize BTX compounds more effectively and efficiently than degradation by an individual isolate of the same consortium, which may be by virtue of the production of different degradative enzymes by mixed microbial population (Leahy et al. 2003).

4.3 Reclamation and ecotoxicity test of bioremediated soil

The crop plants showed better growth performance in bioremediated soil as compared to normal soil. It suggested that the bioremediation of petroleum oil-contaminated soil by seeded bacterial consortium effectively restored the conditions suitable for the germination, growth, and development of crop plants. The better growth performance of Bengal gram and green gram plants in bioremediated soil (Fig. 3) reinforces the idea that residual compounds left out from the contaminating crude petroleum oil are conducive for the growth and development of these crop plants under study. This might be due to the increased levels of nutrients in bioremediated soil as depicted by higher levels of nitrogen, potash, and phosphate (Table 3). The repeated application of low ionic strength liquid culture medium could be a possible reason, but the same was not reflected in the case of phosphorous.

5 Conclusions

The present study vouches that a consortium consisting of three hydrocarbon-degrading bacterial strains, viz. *B. subtilis* DM-04 and *P. aeruginosa* M and NM, can efficiently

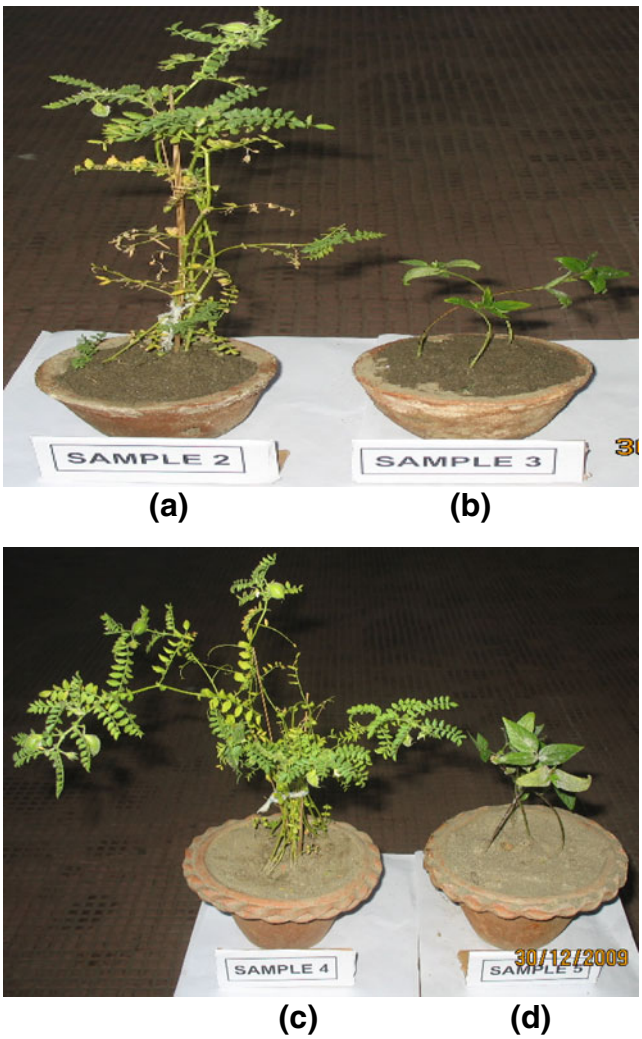

Fig. 3 Growth of Bengal gram and green gram in normal and in reclaimed soil (180 days post-bioremediation). The figure shows Bengal gram in normal soil (**a**), green gram in normal soil (**b**), Bengal gram in bioremediated soil (**c**), and green gram in bioremediated soil (**d**)

degrade components of crude petroleum oil hydrocarbons at a significantly higher rate compared to the degradation of the same compounds by an individual isolate of the consortium. Furthermore, the reclaimed soil supported the germination of seeds and growth of crop plants, thus suggesting the application of bacterial consortium used in the present study for the field-scale bioremediation of petroleum oil-contaminated soil.

6 Recommendations and perspectives

The results achieved in this study advocate the application of bacterial consortium for the field-scale bioremediation of petroleum oil-contaminated soil. Reclaimed soil has shown a low level of ecotoxicity and supports the growth of crop plants, which is highly encouraging because most of the petroleum oil hydrocarbon contaminations in Assam and adjoining states occur in cultivated areas. Since the bioremediation process is an eco-friendly and cost-effective alternative treatment, therefore field-scale experiment using bacterial consortium used in the present study may be recommended for the effective bioremediation of petroleum oil-contaminated soil.

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References

Das K, Mukherjee AK (2005) Characterization of biochemical properties and biological activities of biosurfactants produced by *Pseudomonas aeruginosa* mucoid and non-mucoid strains. J Appl Microbiol Biotechnol 69:192–195

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

Das K, Mukherjee AK (2007b) Comparison of lipopeptide biosurfactants production by *Bacillus subtilis* strains in submerged and solid state fermentation systems using a cheap carbon source: some industrial applications of biosurfactants. Process Biochem 42:1191–1199

Dyreborg S, Arvin E, Broholm K (1989) Effects of creosote compounds on the aerobic bio-degradation of benzene. Biodegradation 7:191–201

Ghazali FM, Rahman RNZA, Salleh AB, Basri M (2004) Biodegradation ofhydrocarbons in soil by microbial consortium. Int Biodeterior Biodegrad 54:61–67

Hornick SB, Fisher RH, Paolini PA (1983) Petroleum wastes. In: Parr JF, Marsh PB, Kla JM (eds) Land treatment of hazardous wastes. Noyes Data Corp., Park Ridge, pp 321–337

Jean JS, Wang Lee MK, Chattopadhyay SM, Maity JP (2008) Effects of inorganic nutrient levels on the biodegradation of benzene, toluene, and xylene (BTX) by *Pseudomonas* spp. in a laboratory porous media sand aquifer model. Bioresour Technol 99:7807–7815

Johnson SJ, Woolhouse KJ, Prommer H, Barry DA, Christofi N (2003) Contribution of anaerobic microbial activity to natural attenuation of benzene in groundwater. Eng Geol 70:343–349

Kim SJ, Choi DH, Sim DS, Oh YS (2005) Evaluation of bioremediation effectiveness on crude oil-contaminated sand. Chemosphere 59:845–852

Kjeldahl J (1883) A new method for the determination of nitrogen in organic matter. J Anal Chem 22:366

Leahy JG, Tracy KD, Eley MH (2003) Degradation of mixtures of aromatic and chloroaliphatic hydrocarbons by aromatic hydrocarbon degrading bacteria. FEMS Microbiol Ecol 1458:1–6

Mattingly GEG (1970) Total phosphorus contents of soils by perchloric acid digestion and sodium carbonate fusion. J Agric Sci 74:79–82

Mukherjee AK, Das K (2005) Correlation between diverse cyclic lipopeptides production and regulation of growth and substrate utilization by *Bacillus subtilis* strains in a particular habitat. FEMS Microbiol Ecol 54:479–489

Obayori OS, Adebusoye SA, Adewale AO, Oyetibo GO, Oluyemi OO, Amokun RA, Iiori MO (2009) Differential degradation of crude oil (Bonny light) by four *Pseudomonas* strains. J Environ Sci (China) 21:243–248

Okoh AI, Trejo-Hernandez MR (2006) Remediation of petroleum hydrocarbon polluted systems: exploiting the bioremediation strategies. African J Biotechnol 5:2520–2525

Petukhov VN, Fomchenkov VM, Chugunov VA, Kholodenko VP (2000) Plant biotests for soil and water contaminated with oil and oil products. Appl Biochem Microbiol 36:564–567

Rahman KSM, Rahman TJ, Kourkoutas Y, Petsas I, Marchant R, Banat IM (2003) Enhanced bioremediation of *n*-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients. Bioresour Technol 90:159–168

Vezquez S, Nogales B, Ruberto L, Hernandez E, Christic-Oleza J, Lo balbo A, Bosch R, Lalucat J, Mac Cormack W (2009) Bacterial community dynamics during bioremediation of diesel oil-contaminated Antarctic soil. Microb Ecol 57:598–610

Wang X, Wang Y, Dai X, Wang L, Han S (2002) Response-surface analysis for the inhibition toxicity of benzene derivatives to yeast *Saccharomyces cerevisiae*. Bull Environ Contam Toxicol 69(2):278–285

Wang l, Qiao N, San F, Shao Z (2008) Isolation, gene detection and solvent tolerance of benzene, toluene and xylene degrading bacteria from near shore surface water and Pacific Ocean sediment. Etremophiles 12:335–342

Wiesel I, Wuebker SM, Rehm HJ (1993) Degradation of polycylic aromatic hydrocarbons by an immobilized mixed bacterial culture. Appl Microbiol Biotechnol 39:110–116