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Enhanced Biodegradation of Oil Products by Some Microbial Isolate Supplemented with Heavy Metals

[1]Aniruddha Sarma and [2]Hemen Sarma
[1]Department of Biotechnology, Pandu College, Guwahati, Assam, India
[2]Department of Botany, School of Life Sciences, North-Eastern Hill University, Shillong, India

Abstract: Three native strains, identified as *Staphylococcus* sp., *Acinetobactor lwoffii* and *Enterobacter agglomerans* were isolated from crude oil contaminated field and the aim of this research was to select potential soil microbial strain that could be effective in the bioremediation of crude oil compounds. Crude oil degradation performed by the isolates incubated in the shake flask culture and analysis of the results address that *Acinetobactor lwoffii,* accelerated cleanup most effectively, degrading oil sludge by a total of 93.78% in compare to *Staphylococcus* sp. (17.39%) and *Enterobacter agglomerans* (16.49%). Furthermore enhanced biodegradation potential of the isolates were studied by adding Mn and Cu and results indicated that supplemented metal increased degradation of crude oil products. In the present study, three bacterial strains AS1, AS2 and AS3 were termed and isolated through long cultivation with crude oil as the single carbon source. The three strains were identified based on the morphology of their colonies with physiological and biochemical characteristics. In addition, the characterizations of soil where the presence of these strains were carried out. The total CFU count ranged from 2×10^6 to 6×10^6 and total viable bacteria at 32°C ranged from 2.2×10^3 to 5.6×10^3 in various soil sample collected in random from contaminated site. The strains had broad degradation capacities and the present remediation monitoring confirmed the effectiveness of *Acinetobactor lwoffii* has one of the potential native microbes for remediation of crude oil soil.

Key words: Soil contamination, petroleum hydrocarbons, bacterial consortium, crude oil, oil sludge reducing bacteria, soil ecosystem, heavy metals

INTRODUCTION

Contamination of soil and sediments with petroleum hydrocarbons is a serious ecological problem, primarily in countries that produce, transport and refine crude oil. Oil production activities release a large amount of hydrocarbon in terrestrial and aquatic environment. The conflict between biosphere and technosphere has sharply increased. The level of soil pollution by petroleum products and oil sludge has approached millions of cubic meters (Zukauskaite and Viktorija, 2008).

Petroleum hydrocarbons viz., saturates, aromatics, asphaltenes (phenols, fatty acids, ketones, esters and porphyrins) and resins (pyridines, quinolines, carbazoles, sulfoxides and amides) usually contaminated soil. Researchers reported that degradation rates have been shown to be highest for the saturates, followed by the light aromatics, with high molecular weight aromatics and polar compounds exhibiting extremely low rates of degradation. Crude oil is a complex mixture of hundreds of different hydrocarbon compounds that widely vary in their characteristics and remediation. Biodegradation of petroleum hydrocarbon is limited to the upper soil profile (Schwab *et al*., 1999). The nutrient addition and frequent aeration of oil contaminated soil are found to be effective in reducing the concentrations of hydrocarbon pollutants (Balba *et al*., 1998). The degradation rates usually decline after a period of time, especially for polycyclic aromatic hydrocarbon compounds (PAHs) (Schwab *et al*., 1999). The physico-chemical methods of cleansing petroleum hydrocarbon are usually expensive and involve the risk of spreading and leaching.

The microbial community has the potential to detoxify hazardous organic compounds through transformation, mineralization, or polymerization. In order to develop a strategy for microbial degradation of crude oil, it is necessary to isolate specific native microbes and to test the efficacy of the microbes in degradation of various hydrocarbon compounds present in oil contaminated site prior to application to the field (Janiyani *et al*., 1993).

Corresponding Author: Hemen Sarma, Department of Botany, School of Life Sciences, North-Eastern Hill University, Shillong 793 022, India Tel: + 91-9774108718

In recent times more emphasis is being given to plant-assisted bioremediation in abating hydrocarbon pollutants in soil. Plants stimulate growth of microorganisms in rhuizospheric region through the release of root exudes. Recent studies have shown that a variety of plants can be used as a polishing step to enhance the degradation of residual compounds.

Potential hydrocarbon degrading bacteria was enlisted by many researchers (Patel and Gosh, 2003; Deka, 2001; Murugesh and Kumar, 2002) and numbers of *Pseudomonas* strains were isolated from oil field of western India. In Nigeria, Oboh *et al.* (2006) isolated naphthalene, kerosene and diesel degrading strains of *Pseudomonas stut* and *Pseudomonas mulli* from Bitumen (Trasand) deposit site. Biodegradation of hydrocarbon has been achieved in Tundra region by using cold adapted microbial consortium (Mohn *et al.*, 2001). Certain other groups of microorganism viz. Proteobacteria has been found to be associated with pyrene degradation in polycyclic aromatic hydrocarbon compounds (PAH) contaminated soil (Singleton *et al.*, 2006). Despite best efforts suitable microbial populations that have the ability to metabolize chemicals compound may not develop at all or may not be present in sufficient numbers to be effective. Often, complicated microbial consortia may be required to biodegrade complex contaminant mixtures such as total petroleum hydrocarbon (TPH) and oil and grease (Cookson, 1995). The ability to degrade and/or utilize hydrocarbon substrates is exhibited by a wide variety of bacterial strains. Floodgate (1984) had enlisted 25 genera of hydrocarbon-degrading bacteria which have been isolated from the marine environment; a similar compilation made by Bossert and Bartha (1984) for soil isolates includes 22 genera of bacteria. Based on the number of published reports, the most important hydrocarbon-degrading bacteria in both marine and soil environments are *Achromobacter*, *Acinetobacter*, *Alcaligenes, Arthrobacter*, *Bacillus*, *Flavobacterium*, *Nocardia and Pseudomonas* sp.

The objective of this study was to isolate the potential native bacterial strains from crude oil contaminated site of Joipur oil field of Oil India Ltd., (Duliajan), Assam, India and study the efficacy of these strains for bioremediation of a crude oil compounds. The other objective includes consortia effect of the isolates on the degradation of crude oil, study the characteristics of the soil where from the native strain were isolated and how these strains are more efficient in the presence of heavy metal and what are the novelty of these strains in crude oil degradations.

MATERIALS AND METHODS

Site description: The field site is located at Joipur oil field, Oil India limited in South-Western Assam (27°21'37.25" N and 95°19'32.11" E) India. This region is characterized by a hot sub-humid (moist) to humid (inclusion of per-humid) climate with alluvial dried soil. Mean daily temperatures range from 23°C in January to 32°C in July and precipitation averages 2332 mm year^{-1}. The site covers approximately 5 hectare and the soil consists of a granular fill approximately 0.15 to 0.30 m in depth overlying a clay, silt and sand. This site was currently used for tank storage of crude oil and transportation, which gave rise to hydrocarbon contamination of the soil. The study accomplished collection of soil samples from the site where hydrocarbon pollution was evident and isolation of bacteria species in a series of experiments.

Soil collection and chemical and physical analyses: Soil (J#1, J#2, J#3, J#4) were collected from contaminated sites at Joipur oil field in August 2007 and stored at 4°C before being transported to Institute of Advanced Study in Science and Technology, Guwahati, for analysis. The soils were processed for immediate microbiological analyses. For comparison, a non-contaminated reference soil with similar soil characteristics was collected from a region ~1 km outside of Joipur oil field, Duliajan, Assam. Total crude oil was extracted from the soil samples by solvent extraction methods. Sludge (50 g) + Dichloromethane (300 mL) were taken and stirred well for 1 h at 300 rpm. Replicate oil extraction was carried out to minimize the error. All the aliquot was distilled to retain DCM and remaining crude oil was stored at 4°C. Standard soil analytical techniques were used to determine the particle size, water holding capacity, soil pH, organic carbon and nitrogen of the soil (Trivedi and Sudarshan, 1994).

Indigenous bacterial species isolation: The contaminated soils were prepared as soil slurries to obtain highly active hydrocarbon-degraded inoculums, consisting of indigenous microorganisms. The soil slurries consisted of 1 g of a soil (each soil J#1, J#2, J#3 and J# 4) were taken aseptically with 99 mL of sterile water and shaken in a water bath (32°C) shaker at 150 rpm. All incubations were performed at 32°C. The bacterial populations were estimated by minimal media (Bushnell-Hass medium) using crude petroleum oil as the single carbon source through serial dilution methods up to 10^{-6}. The media was plated and 1 mL of soil sample solution from 10^{-4} and 10^{-6}

were inoculated and incubated for 72 h. Colonies developed in the plates were re-cultured in nutrient agar plate in streak plate method and incubated for 48 h and bacterial stains were isolated in test tube slant containing nutrient agar. In the present study, highly active indigenous petroleum crude oil degrading consortia were also prepared and crud oil degradation activity test were done at 32° C after 72 h of incubation.

Petroleum crude oil degradation study-shake flask culture: The biodegradation of petroleum crude oil in the oil field soils was determine through shake flask culture methods and effectiveness of each strain were studied. Treatment (Step I) was designed to know the amount of crud oil degradation by the isolated strain: Step I, Broth (100 mL) supplemented with + 2 g semi liquid crude oil as carbon source and sterilized; Treatment (Step II) was designed to enumerate bacterial isolates for their efficacy in crude oil degradation: Step II, isolated bacterial species were inoculated aseptically with five replications of each isolate. Step III, isolated bacterial species were inoculated aseptically with five replications (2 g crude oil + 100 mL broth+1000 ppm Cu). Step IV, isolated bacterial species were inoculated aseptically with five replications (2 g crude oil + 100 mL broth+10 ppm Mn). Step V, bacterial consortia were inoculate with five replication of each with or without metal (Cu, Mn) +2 g crude oil + 100 mL broth. A control (2 g crude oil + 100 mL broth) was maintained without addition of bacterial colony. All incubation was done at 32°C in a refrigerated shaking incubator up to 180 days at 150 rpm. The specific crude oil catabolic bacterial colonies were isolated from the cultural flask and transferred for pure culture for enumeration of isolates. Furthermore the degradation of crude oil also measured by extracting with DCM after the periodic interval during the incubation period to know the amount of residual crude oil left in the experimental flasks.

Petroleum crude oil degrading activity test: The dynamics of petroleum crude oil biodegradation by the indigenous microbial culture in soil under aerobic condition was determined in 250 mL flasks containing 20000 ppm of crude oil. The experimental flasks had two side arms, tightly closed with Teflon®-lined caps in order to prevent evaporation of hydrocarbons and to minimize their loss through adsorption onto the caps. The experiments were performed at 32°C in order to simulate summer conditions at the contaminated site. The activity tests were performed in replicates. The experimental flasks were incubated for 180 days with shaking at 150 rpm. Samples were withdrawn at predetermined intervals and were analyzed for their content of total petroleum crude oil.

Enumeration and biochemical characterization of indigenous bacterial isolates: Total viable bacteria (i.e., culturable organisms) in the soil samples were enumerated on Mineral Salts Medium (MSM) containing 250 mg L^{-1} each of yeast extract, tryptone and starch (YTS) at 32°C (mesophilic populations) (Greer *et al*., 1990). Total bacteria in the soil samples were directly enumerated by fluorescent staining with 5-(4, 6-dichlorotriazine-2-yl) aminofluorescein method (Bloem, 1995) and biochemical characterization were done at IMTech, Chandigarh. In this study, we used a normal category method (Du, 1992) to identify the genera and genus of the bacterial strains based on the morphological, physiological and biochemical characteristics.

RESULTS AND DISCUSSION

Soil chemical and physical analyses and microbial count: The contaminated soils possessed similar soil characteristics with the exception of their oil concentrations (Table 1). Crude oil was not detected in the control soil but varied from very low concentrations in J#1(145.8 mg kg^{-1}) to high concentrations in J#3 (1237 mg kg^{-1}). Adequate water holding capacity (WHC) existed in the soils, the pH of the soils was acidic and organic carbon (OC) appeared in the soil and ranged vary from 22.78-54.21 g kg^{-1}. The physico-chemical characteristics of the soil and the bacteria strains (petroleum crude oil degrading) showing no correlation as bacterial isolates were flourished well within the pH range (4.98-5.5). This finding is contradictory with the earlier findings that pH (7.64-8.85) is optimal for diesel degradation (Oboh *et al*., 2006). The difference in pH can be attributed to the fact that pH is always a determining factor for abundance of soil micro-flora and soil characteristic is important factor for population dynamics as well as degradation of crude oil (Deka, 2001). The presence of microbial activity in soil was determined by enumeration of total heterotrophic bacteria, growing on Bushnell-Hass medium followed by colony count after 72 h incubation at 32°C. The total CFU count 2×10^6 in J#1, 6×10^6 J#2, 3×10^6 in J#3 and 4×10^6 in J#4 total viable bacteria at 32°C was 2.2×10^3 in J#1, 5.6×10^3 in J#2, 3.4×10^3 in J#3 and 5.6×10^3 in J#4 . Crude oil utilizing bacterial consortiums was present in abundance at vary site of oil seepages (Mikesell *et al*., 1993). Total N concentrations

Table 1: Soil property values for the 4 sampling sites at Joipur oil field (0-25 cm soil layer)

Soil sample	Total OC (g kg^{-1})	Total N(g kg^{-1})	C: N ratio	pH	Crude oil (mg kg^{-1})	WHC (%)	Soil texture (%)		
							Silt	Clay	Sand
Control	22.78±0.64	1.48±0.20	15.39	5.80	Nil	58.87	33.581	13.908	52.511
J#1	54.21±1.91	2.51±0.53	21.59	4.98	145.8	48.64	43.798	3.008	53.194
J#2	48.45±0.16	2.37±0.24	20.44	4.93	239.7	56.50	55.990	10.81	33.2
J#3	35.51±0.29	2.1±0.140	16.90	5.09	1237.0	39.87	0.002	10.814	89.184
J#4	42.78±0.74	2.3±0.150	18.60	5.50	504.6	50.59	36.006	10.806	53.188

were significantly greater in the 1.48 to 2.51 g kg^{-1} across the site (Table 1), with mean total N value 2.32 g kg^{-1} and soil C:N ratios across the site ranged from 15.39 to 21.59 (Table 1). Overall, ratios were significantly different all the sampling site and the highest ratios were observed in the J#1 and J#2. The nutrient status of the soil has been altered by petroleum hydrocarbon and in turn affect the ability of microorganisms to reclamation of contaminated site (Riser-Roberts, 1998). Therefore, understanding the relationships between soil nutrient status and total crude oil concentrations is important for microbial remediation of crude oil contaminated site. The highest residual petroleum crude oil concentration measured at Joipur#3 soil layer (0-25 cm) was 1237.0 mg kg^{-1} (Table 1). Nutrient status and organic matter content are important factors affecting petroleum hydrocarbon degradation in the soil (Young and Mulligan, 2004). The addition of petroleum hydrocarbons to the soil generally increases the C:N ratio (Xu and Johnson, 1997). Xu and Johnson (1997) suggested that ratios less than 25:1 lead to mineralization and ratios greater than 30:1 lead to immobilization. Immobilization can severely limit bio and phyto-degradation of organic contaminants due to insufficient N being available for metabolism of contaminants (Riser-Roberts, 1998). In particular that the ratios measured at the Joipur site were all below 30:1 (Table 1), the N content of the soil should be sufficient to allow widespread microbial degradation of hydrocarbons. The highest C:N ratios were found in J#1 and J#2, which, surprisingly, corresponded with low petroleum crude oil concentrations (Table 1). High C: N ratios in J#1 and J#2 may results from increased rates of mineralization leading to N depletion. The bioavailability of petroleum hydrocarbons in soil for bioremediation decreases as the organic carbon content of the soil increases (Weissenfels *et al*., 1992). This results mainly from hydrophobic partitioning of the hydrocarbon compounds onto humic substances and diffusion of compounds into soil-humus matrices (Hwang *et al*., 2002). Murphy *et al.* (1990) reported that sorption of hydrophobic organics onto humic acid coated soil was nonlinear; rates were initially rapid but eventually reached equilibrium.

Enumeration and biochemical characterization of indigenous microorganisms: Microbial enumeration exhibited that significant microbial populations are present in the entire soil sample as shown by CFU counts and total viable counts (cultivable heterotrophic bacteria). As expected, the CFU counts typically were higher (~2×10^6 to 6×10^6) in the soil (J#1, J#2, J#3 and J# 4) and all were mesophilic populations. The optimal UV florescence growth is 15-37°C and based on the biochemical composition (Table 1, 2) the specific petroleum crude oil catabolic strains have been identified as AS1 (*Staphylococcus* sp.), AS2 (*Acinetobactor lwoffii*) and AS3 (*Enterobacter agglomerans*). It was found that the colonies were round, entire, convex, smooth and translucent with no pigmentations; AS1 were gram positive and had no endospores; the cells were shaped like cocci. For the colonies of AS2 and AS3, it was found that they were round, gram negative and had no endospores; the cells were shaped like rods. The biochemical profiles of the designated bacterial isolates (AS1, AS2 and AS3) were studied using various test and their responses were summarized in Table 3.

Assessment of biodegradation potentials of isolates: Petroleum crude oil degradation capacities of *Staphylococcus* sp., *Acinetobactor lwoffii* and *Enterobacter agglomerans* were studied through activity tests. The activity tests were carried out without bioaugmentation and the biodegradation activities were attributed to the indigenous microbial isolates in the contaminated soil. The three bacterial isolates had a various capacity for biodegradation of petroleum hydrocarbons. After 180 days of incubation, the residual substrates were measured and the results are presented in (Table 4). *Acinetobactor lwoffii* showed a remarkable potential, as it was able to degrade maximum crude oil representing a maximum removal efficiency of 93.78% after 180 days of incubation in compeer to *Staphylococcus* sp. (17.39%) and *Enterobacter agglomerans* (16.49%). Overall, the results from these three isolates, *Acinetobactor lwoffii* have maximum biodegradation potential of the target compounds. Although, we do not

Table 2: Morphological and physiological characteristics of designated bacterial isolates

	Designated strains		
Tests	AS1	AS2	AS3
Colony morphology			
Configuration	Round	Round	Round
Margin	Entire	Entire	Entire
Elevation	Convex	Convex	Convex
Surface	Smooth	Smooth	Smooth
Density	Translucent	Translucent	Translucent
Pigment	-	-	-
Gram's reaction	+ ve	- ve	- ve
Shape	Cocci	Rods	Rods
Size	Small	Short	Short
Arrangement	Single and group	Single	Single
Spore/Endospore	-	-	-
Motility	+	+	+
Fluorescence (uv)	-	-	-
Growth at temp.			
4°-10°C	-	-	-
15°-37°C	+	+	+
42°- 65°C	-	-	-
Growth at pH			
5.00-11.0	+	+	+
Growth on NaCl (%)			
2.5-7.0	+	+	+
8.5	+	-	±
10	+	-	-
Growth under anaerobiccondition	+	-	+

-: Absence, +: Absence

Table 3: Biochemical profile of the designated bacterial isolates

	Designated strains		
Tests	AS1	AS2	AS3
Growth on MacConkey agar	-	+	+
Indole test	-		
Methyl Red test	-	-	-
Voges Proskauer test	-	-	±
Citrate utilization	-	+	+
Gas production from glucose	-	-	+
Casin Hydrolysis	-	+	+
Starch hydrolysis	-	-	-
Urea hydrolysis	-	-	-
Nitrate reduction	+	-	+
Nitrite reduction	±		
H_2s production	-	-	-
Cytocrome oxidase	-	-	-
Catalase test	+	+	+
Oxidation/Fermentation	F	-	F
Gelatin hydrolysis	+	±	+
Arginine hydrolase	+	+	+
Lysine decarboxilase	-	-	-
Orithine decarboxilase	-	-	-
Adonitol	-	-	-
Arabinose	-	-	+
Cellobiose	-	-	+
Dextrose	+	-	+
Dulcitol	-	-	-
Fructose	+	-	-
Galactose	-	-	-
Inositol	-	-	-
Lactose	-	-	+
Maltose	+	-	+
Mannitol	-	-	+
Melibiose	-	-	-
Raffinose	-	-	-
Rhamnose	-	-	+
Salicin	-	±	-
sorbitol	-	-	-
Sucrose	+	-	-
Trehalose	-	-	-
Xylose	-	-	+

-: Absence, +: Absence

have a conclusive explanation about the remarkable potential of *Acinetobactor lwoffii* in terms of petroleum crud oil removing efficacy but we presume that various intrinsic and extrinsic factors may be involved, namely physiology, temperature and metabolism. However, treatment with the microbial consortia appreciably reduces crud oil concentrations to a greater extent. In this study co culture with three strain we have recorded a maximum of 96.63% crud oil degradations reinforcing the earlier findings of Cookson (1995) that complicated microbial consortia required to biodegrade complex contaminant mixtures such as total petroleum hydrocarbon (TPH) and oil and grease. Aromatic hydrocarbons such as benzene, toluene, ethyl-benzene and xylene (BTEX) are classified as environmental priority pollutants found together in crude petroleum and petroleum products such as gasoline and diesel fuel. A newly isolated strain from water treatment plant in Egypt was identified as *Pseudomonas* sp. H12 and this strain is highly efficient in degrading hydrocarbon mixture BTXHB (Ranya Amer *et al*., 2008) and this is confirmatory with the present findings that native bacterial isolates has remarkable potential to bidegrade complex contaminant mixture of hydrocarbon.

Temperature influences petroleum hydrocarbon biodegradation by its effect on the physical nature and and chemical composition of the oil, rate of hydrocarbon metabolism by microorganisms and composition of the microbial community (Atlas, 1981). At low temperatures, the viscosity of the oil increases, the volatilization of toxic short-chain alkanes is reduced and their water solubility is increased, delaying the onset of biodegradation. Higher temperatures increase the rates of hydrocarbon metabolism to a maximum, typically in the range of 30 to 40°C, above which the membrane toxicity of hydrocarbons is increased (Bossert and Bartha, 1984). However, thermophilic alkane utilizing bacteria do exist.

Nitrogen and phosphorus may also play an important role in the acceleration of the biodegradation of crude oil or gasoline in soil and groundwater. The addition of urea-phosphate, N-P-K fertilizers and ammonium and phosphate salts has been demonstrated in several studies (Dibble and Bartha, 1979). Other investigators observed an increase only after a delay of several months to a year

(Odu, 1987) when fertilizer amendments were used in bio-remedation of oil products.

The influence of heavy metals on microbial activities, both separate and synergetic, has been widely discussed (Khan and Scullion, 1999; Witter *et al*., 2000). Heavy metals are toxic to most organisms when present in high concentrations in the environment (Dai *et al*., 2004). However, in some cases their low concentrations can have a positive effect (due to catalytic abilities) on speeding up the fermentation process. Heavy metals can also affect the growth, morphology and metabolism of micro-organisms in soil.

The residual concentration of petroleum crude oil left after periodic incubations of soil bacterial isolates (AS1, AS2 and AS3 tested alone and co culture) supplemented with Cu and Mn presented in Table 5 and 6. In all samples the residual concentrations of crud oil remarkably decreased after 180 days incubation with added heavy metal (with Mn and Cu) for all the isolates. Statistically, the most significant difference ($p<0.05$) between the isolates was observed after periodic incubation. The results of our experiment indicate that using Mn and Cu the effectiveness of crude oil degradation can be enhanced in the isolated strain and our results improved the knowledge of biodegradation of crude oil and laid an important foundation for further development of advanced bioremediation strategies.

Biostimulation of indigenous microbial populations with metals amendments is attractive *in situ* bioremediation strategies because such treatments can increase contaminant degradation rates. Certain bacterial consortiums are now-a-days regularly used to clean the oil-spill after transporting large quantities of crude oil with the help of carbon minimal medium like Bushnell-Hass medium (Madigan *et al*., 1997). In bioremediation process growth and survival of microorganism is affected by environmental factors like temperature, composition of contaminant, soil type and nutrient and water availability (Salleh *et al*., 2003). Reductions of pollution by administrating active hydrocarbon degrading strain from the site is become a promising technology. The strains are always site specific and their administration does not impending the risk that is associated with genetically modified strains. The present findings will ascertained that reclamation of petroleum hydrocarbon contaminated oil field (Dibrugarh, Assam) might be possible by application of indigenous strain of bacteria. To the best of our knowledge, *Acinetobactor lwoffii* is the first native pure strain with such broad degradation capacity that has been characterized in detail and can be used for bioremediations of crud oil contaminated sites.

The elimination of contaminating hydrocarbons by indigenous microbial isolates is a significant tool to remove the fraction of oil since it cost effective and

Table 4: Residual concentration of petroleum crude oil (ppm) left after periodic incubations of soil bacterial isolates

Bacterial isolates	Initial concentration of crude oil	Residual concentration after incubation			Removal (%) of crude oil after 180 days
		90 days	135 days	180 days	
AS_1	20,000	17855 ± 18^a	17154 ± 25^a	16522 ± 15^a	17.39
AS_2	20,000	5431 ± 22^b	3648 ± 34^b	1244 ± 46^b	93.78
AS_3	20,000	17921 ± 13^c	17317 ± 11^c	16702 ± 13^c	16.49
Consortia effect	20,000	1157 ± 13^d	895 ± 13^d	674 ± 13^d	96.63

Different superscripts denote significant ($p<0.50$) difference between the means in a column

Table 5: Residual concentration of petroleum crude oil (ppm) left after periodic incubations of soil bacterial isolates supplemented with Cu

Bacterial isolates	Initial concentration of crude oil	Residual concentration after incubation			Removal (%) of crude oil after 180 days
		90 days	135 days	180 days	
AS_1	20,000	12845 ± 18^a	11157 ± 25^a	10562 ± 15^a	47.19
AS_2	20,000	3561 ± 22^b	2618 ± 34^b	1461 ± 46^b	92.69
AS_3	20,000	14625 ± 13^c	12314 ± 11^c	11602 ± 13^c	41.99
Consortia effect	20,000	1157 ± 13^d	895 ± 13^d	581 ± 13^d	97.09

Different superscripts denote significant ($p<0.50$) difference between the means in a column

Table 6: Residual concentration of petroleum crude oil (ppm) left after periodic incubations of soil bacterial isolates supplemented with Mn

Bacterial isolates	Initial concentration of crude oil	Residual concentration after incubation			Removal (%) of crude oil after 180 days
		90 days	135 days	180 days	
AS_1	20,000	11626 ± 18^a	10177 ± 25^a	9572 ± 15^a	52.14
AS_2	20,000	3871 ± 22^b	2919 ± 34^b	1481 ± 46^b	92.59
AS_3	20,000	13421 ± 13^c	12614 ± 11^c	11402 ± 13^c	42.99
Consortia effect	20,000	1057 ± 13^d	995 ± 13^d	454 ± 13^d	97.73

Different superscripts denote significant ($p<0.50$) difference between the means in a column

environment friendly. Although, the process exhibited an enhanced removal of contaminating pateroliem hydrocarbons, further studies are still needed in order to optimize the process and assess its applicability. Generally, a compound is biodegraded if favourable environmental conditions can be created that encourage the growth of certain microorganisms that have the ability to metabolize. In most bioremediation research, great efforts are made to optimize environmental conditions (e.g., moisture, pH, fertilizer content, aeration etc.) in order to stimulate the growth of hydrocarbon-degrading microorganisms (Huesemann, 1994). Consequently, in most cases, the lack of optimal environmental conditions is unlikely to limit the overall biodegradability of total petroleum hydrocarbon (TPH).

CONCLUSIONS

In conclusion, the capacity of three bacterial strains with the ability to degrade petroleum crude oil was determined supplemented with heavy metal. In general Mn and Cu enhanced the biodegradation potentials of the isolates remarkably. To the best of our knowledge, *Acinetobactor lwoffii* is the first native pure strain with such broad degradation capacity that has been characterized in detail. This study also emphasized that complicated microbial consortia required to biodegrade total petroleum crude oil effectively. However, the biodegradation of petroleum and other hydrocarbons in the environment is a complex process, whose quantitative and qualitative aspects depend on the nature and amount of the oil, the ambient environmental conditions and the composition of the microbial community.

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