Original Research Article

Isolation and Screening for Hydrocarbon Utilizing Bacteria (HUB) from Petroleum Samples

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A B S T R A C T

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The wide spread use of petroleum products leads to contamination of soil and aquatic environments, thereby poses a serious threat to all life forms including humans. Bioremediation is considered a cost-effective and environmentally safe method for treating oil polluted sites. Therefore, isolation of oil-degrading microorganisms and optimizing conditions for biodegradation process are important aspects of petroleum microbiology**.** Some important microbial species with this potential belong to the genera *Arthrobacter, Halomonas, Pseudomonas*, *Bacillus*, *Klebsiella, Proteus*, *Aspergillus, Neurospora, Rhizopus*, *Mucor, Trichoderma etc.* Microbial species differ in their biodegradation capabilities; therefore screening of potential hydrocarbon utilizing organisms is an essential process. Enrichment culture technique was employed to obtain sixty (69) bacterial isolates capable of utilizing crude oil as a carbon source from various soil and untreated effluent water (UEW) samples collected from various oil fields located in Gujarat. The average density of hydrocarbon utilizing bacteria (HUB) relative to the total viable count (TVC) of heterotrophic bacteria in the soil samples was 27.6 %, while in UEW samples was 15.8%. Screening for bacteria utilizing crude oil as the sole source of carbon with 2, 6-dichlorophenol indophenol (DCPIP) as redox indicator was carried out for all the isolates. Based on their capability to degrade hydrocarbons, six isolates were further selected and mixed to prepare a consortium which showed maximum utilization of hydrocarbons indicated by the total discoloration of DCPIP in just 53 hours. The results of this study are presented here.

Introduction

Hydrocarbons are organic compounds which are composed of two main elements-carbon and hydrogen. They also contain small quantities of molecules containing sulphur, nitrogen, metals, oxygen, *etc*., (Vieira *et al*., 2007).

Petroleum, in Latin means "*rock oil*", which occurs as a dark, sticky, viscous liquid. Petroleum products such as gasoline, kerosene, diesel / fuel oil, crude oil are complex mixture of organic compounds basically of paraffinic,

oleifinic and aromatic hydrocarbons (Mittal and Singh, 2009; Singh and Lin, 2008; Vieira *et al.*, 2007). One of the major environmental problems today is hydrocarbon pollution by petrochemical industry (Sebiomo *et al.,* 2010), widespread release of aromatic hydrocarbons through spillages and leakage from underground tanks, steamers causing extensive contamination of surface soils, groundwater, seas and oceans (Bayoumi and Abul-Hamd, 2010).

Microbial remediation of hydrocarbon-contaminated site is performed with the help of a diverse group of microorganisms, particularly the indigenous bacteria present in soil (Sebiomo *et al*., 2010). The first line of defense against oil pollution in the environment is the microbial population (Youssef *et al*., 2010).

Bioremediation is emerging as one of the most promising technologies for the removal of petroleum hydrocarbons from the environment. For this, the screening of potential crude oil degrading organisms is one of the key steps. Screening for HUB was carried out by DCPIP method as described earlier (Bidoia *et al.,* 2010l; Hanson *et al.,* 1993). This paper deals with sampling, chemical analysis, quantitative bacteriological analysis, isolation and screening of HUB from crude oil, soil and UEW samples from various oil-fields of ONGC located in Gujarat. It also deals with screening for quantitative and qualitative biodegradation potential of the pure cultures of selected bacterial isolates as well as their consortium by DCPIP method.

Materials and Methods

Sample collection

Samples were collected with the technical assistance of ONGC authorities from various oil fields of ONGC sites located in Gujarat as described later. Total six subsurface soil samples (1.0 kg each) were collected near oil wells, Gas Gathering Stations (GGS) and Central Tank Farm (CTF) in sterilized plastic bags from Ankleshwar (Ank), Ahmedabad 1 (AH 1) and Ahmedabad 2 (AH 2) fields. Total five UEW samples (2.0 liter each) were collected from GGS and CTF in pre-sterilized plastic carbides from Ank, AH 1, AH 2 and 52 Ahmedabad 3 (AH 3) fields. Total six crude oil samples (2.0 liter each) were collected from the well mouth of oil wells, GGS and CTF in sterile plastic carbides from AH 1, AH 2 and AH 3 fields. Samples were labeled and transported to the laboratory and stored in at 4ºC before analysis.

Physico-chemical characteristics of soil

Soil samples were analyzed for pH, moisture content, specific gravity, dry density, grain size, total organic carbon (TOC), nitrogen and phosphorus, oil content and metal composition. The pH and metal composition were determined by digital pH meter (Systronics µ, India, model 361) and standard atomic absorption spectrophotometer (Elico, Sl 194, India), respectively.

Moisture content, specific gravity, dry density and grain size were analyzed by ASTM D 2216, ASTM D 854, IS: 2720 (Part XXIX - 1975) and ASTM D 422, respectively.

TOC, nitrogen, phosphorus and oil content were determined as reported previously (Mittal and Singh, 2010; Obayori *et al.,* 2012).

Physico-chemical characteristics of UEW

UEW samples were analyzed for pH, turbidity, ionic composition, salinity and TDS. The pH and turbidity were determined by digital pH meter as above and Micro 100 IR turbidimeter (HF Scientific), respectively. Ionic composition, salinity and TDS were determined as reported previously (Adebusoye *et al.,* 2008; Vogel, 1962).

Physico-chemical characteristics of crude oil

Crude oil samples were subjected to SARA (Saturates, Aromatics, Resins and Asphaltenes) analysis. SARA analysis was performed by TLC-FID detector (HYDROSCAN MK-6s) following IP 469. On the basis of paraffin content two samples namely Ank CTF and K#X (table 4a), were selected and tested for physico-chemical parameters viz. density (DMA 48), API gravity, viscosity (Brook field viscometer) and pour point (Autopour 300) (Mittal and Singh. 2009]. Crude oil sample from K#X showed presence of resins as well as asphaltenes but asphaltenes were not detected in crude oil sample from Ank CTF. Hence further studies of crude oil utilization were carried out using sample from well K#X.

Quantitative bacteriological analysis Determination of TVC

TVC of heterotrophic bacteria from collected soil and UEW samples was determined by serial dilution method

(Hamzah *et al.*, 2010; Youssef *et al*., 2010). Ten grams of each soil sample was added to 90 ml sterile normal saline (0.90% w/v, NaCl) and mixed by vortexing. Aliquotes of 10 ml UEW sample(s) was diluted in 90 ml sterile normal saline. Then 0.1 ml of the serially diluted soil and UEW samples was plated on Nutrient agar medium and incubated at 37 ± 2ºC for 24 - 48 h. Bacterial count was determined by CFU per g or ml of respective samples.

Determination of HUB

Density of HUB from the samples was determined by serial dilution method and dilutions were prepared as described earlier. Aliquotes of 0.1 ml from serially diluted soil and UEW samples were plated on Bushnell-Haas (BH) medium and incubated at 37 ± 2ºC for 24 - 48 h. HUB count was determined by CFU per g or ml of respective samples. The composition of BH medium (g/liter): 0.2 g $MgSO_4$, 0.02 g $CaCl_2$, 1.0 g KH_2PO_4, 1.0 g K_2HPO_4, 1.0 g NH_4NO_3, 0.05 g $FeCl_3$, and the pH was adjusted to 7 - 7.2 with 0.01N HCl (Xia *et al*., 2006: Youssef *et al*.,2010).

Percentage of HUB in TVC

Percentage of HUB in TVC of heterotrophic bacteria was calculated by following formula:

Percentage of HUB in TVC= $(HUB\times100)$/TVC (Joshi and Pandey, 2011; Youssef *et al*., 2010).

Enrichment and Isolation

Soil sample (1.0 g) from each source was suspended and vortexed with 10 ml normal saline. The solids were allowed to settle down, one ml supernatant

(inoculum) and 1% crude oil was added to 100 ml of Luria-Bertani (LB) broth in 250 ml Erlenmeyer flask. The flasks were incubated for 48 h at 37±2ºC on a rotary shaker at 180 rpm. Three consecutive sub-culturing was done with same conditions. The third subculture of each sample in LB broth was centrifuged at 5000 rpm for 10 mins. The supernatant was discarded and cell pellets were washed twice with 0.1 M phosphate buffer solution (pH 108 6.8). After centrifugation the pellets were resuspended in 1.0 ml BH medium and Mineral Salt (MS) medium, this culture suspension was inoculated in 100 ml of BH as well as MS broth in 250 ml Erlenmeyer flask containing 1% crude oil (K#X). The crude oil serves as the sole carbon source. The flasks were incubated for 5 - 6 days at 37±2ºC on a rotary shaker at 180 rpm. Sub-culturing was repeated using BH and MS broth as described for LB broth. After third sub-culture pure colonies of HUB were obtained on crude oil agar plates.

The plates were prepared by spreading 1 - 2 drops of sterile crude oil evenly on BH and MS agar plates. The plates were inoculated by spreading 0.1 ml growth from medium in the third sub-cultured flask and incubated at 37±2ºC for one week. Pure cultures of the isolates were maintained on Nutrient agar slants and stored at 4ºC in a refrigerator (Afuwale and Modi, 2012; Mittal and Singh, 2009).

Screening of Hydrocarbon utilizers

Screening of potential HUB was carried out by modified Hanson *et al.,* (1993); Bidoia *et al*., (2010) method using DCPIP as redox indicator. This technique was also employed in other studies (Afuwale and Modi, 2012; Joshi and Pandey, 2011; Mariano *et al*., 2008). During the

microbial oxidation of hydrocarbons, electrons are transferred to electron acceptors DCPIP to the culture medium, and it is possible to ascertain the ability of the microorganism to utilize hydrocarbons by observing the colour change from blue (oxidized) to colorless (reduced), which is monitored at 600 nm wavelength.

Inoculum was prepared by transferring cultures from Nutrient agar slants into BH medium for 24 h at 37±2ºC at 180 rpm. Cultures were then inoculated into tubes along with DCPIP indicator (0.5% w/v) and the selected crude oil (K #X) (3%, v/v) for spectrophotometric analysis. All the tubes were incubated at room temperature for 144 h. Appropriate controls and substrate/crude oil assays were included as mentioned in Table 1. The absorbance of all the assays was measured by Hach® DR 2500 spectrophotometer. Data was collected at regular time interval of 24 h till 144 h.

Preservation, maintenance and sub-culturing

Selected HUB strains were preserved in 20% v/v sterile glycerol solution at -70ºC. For routine experiments isolates were maintained on Nutrient agar slants at 4ºC in refrigerator and sub-cultured at an interval of 30 days.

Results and Discussion

Physico-chemical characteristics of soil

The physico-chemical characteristics of the soil samples collected from various oil-fields are represented in Tables 2a and 2b. The soil samples used in the study had pH range of 6.5 - 7.5 and moisture content varied from 11.02 - 27.12%. Total Organic Carbon (TOC), nitrogen, phosphorous, and

oil content of soil samples were 25 - 30%, 2 - 10%, 0.1 – 4.0% and 6.2 - 7.2% respectively. The results of specific gravity, dry density, grain size and metal composition of the samples were studied (Table 2a and 2b).

Table. 1 Protocol for colorimetric analysis for screening of HUB

Tube no.	DCPIP (μl)	BH medium (ml)	Culture (μl)	Crude oil (μl)
ISC	2000	7.5	-	-
SC	2000	7.5	1000	-
C-HUB$_n$	2000	7.5	-	300
T-HUB$_n$	2000	7.5	1000	300

ISC: Inoculum and substrate control, SC: Substrate control, C-HUB$_n$: Control for respective HUB, T-HUB$_n$: Test for respective HUB

Table. 2a Physico-chemical characteristics of soil

Sr.No	Characteristics	Range
1	pH	6.5 - 7.5
2	Moisture Content (%)	11.02 - 27.12
3	Specific Gravity (kg/m$^{3)}$)	2500 - 2670
4	Dry Density (kg/m$^{3)}$)	1318 -1165
5	Grain size (%)	
	(A) Gravel	12.018 - 36.64
	(B) Sand	29.797 - 63.93
	(C) Fine Content	13.221 - 33.792
6	Nitrogen (%)	2 -10
7	Total Organic Carbon (%)	25 - 30
8	Phosphorus (%)	0.1- 4.0
9	Oil Content (%)	6.2 - 7.2

Table 2b. Physico-chemical characteristics of soil: metal analysis

Zn (ppb)	Mn (ppb)	Cr (ppb)	Cu (ppb)	Ni (ppb)	Pb (ppb)	Co (ppb)	As (ppb)	Cd (ppb)	Se (ppb)
4.31-14.16	18.3-21.45	0.87 – 11.2	0.65- 2.64	0.12-12.86	0.80 – 4.3	0.56 – 0.78	0.3-0.57	BD$^{\times}$	0.1-0.62

(BD – below detection)

Table 3. Physico-chemical characteristics of UEW

pH	Turbidity (NTU)	Ionic Composition (mg/l)							Salinity (mg/l)	TDS (mg/l)
		Cl^-	Ca^{2+}	Mg^{2+}	CO_3^{2-}	HCO_3^{2-}	SO_4^{2-}	Na		
7.2-8.0	29.3 - 1075	137567 -47570	28.07-465.16	14.7-196	0-45	335.5-976	2-6	24503-92708.3	58802-221236	63950-236166

Table. 4b Physico-chemical characteristics of crude oil

Sr. No.	Characteristics	Ank CTF	K #X
1	Density (15.5 ºC) (kg/m^3)	845.1	867.5
2	Specific Gravity (15.5°C) (kg/m^3)	845.9	868.3
3	API Gravity (15.5°C) (°API)	35.77	31.46
4	Viscosity (cp)	623.11	1885.02
5	Pour Point (°C)	18	27
6	Sat/Aro	5.05	6.1429

Physico-chemical characteristics of UEW

The physico-chemical properties of UEW samples as given in Table 3, showed pH value ranging from 7.2 - 8.0, where as salinity (NaCl) and TDS ranging from 58802 - 221236 mg/l and 63950 - 236166 mg/l respectively. The major ions present were Cl^-, Ca^{2+}, Mg^{2+} and HCO_3^{2-} ranging from 137567 - 47570 mg/l, 28.07 - 465.16 mg/l, 14.7 - 196 mg /l and 335.5 - 976 mg/l (Table 3).

Physico-chemical characteristics of crude oil

Crude oil samples contained moisture content, saturates, aromatics, resins and asphaltenes in the range 0 - 24%. 77.2 - 80.8%, 12.4 - 17.8%, 3.2 - 8.8% and 0.8 - 4.3%, respectively (Table 4a). The density, specific gravity, API gravity, viscosity, pour point and Sat/Aro of the selected two crude oil samples out of six samples collected are recorded in Table 4b.

Quantitative bacteriological analysis Determination of TVC

The density of TVC by serial dilution method at 37±2°C is shown in Table 5. It showed that the bacterial cell density in the soil samples fluctuated between 0.126×10^8 - 0.08×10^9 CFU/g, while in UEW samples it fluctuated between 0.105×10^8 - 0.26×10^9 CFU/ml. The average bacterial cell density was 3.86×10^7 CFU/g and 6.66×10^7 CFU/ml in the soil and UEW samples, respectively.

Determination of HUB

HUB count by serial dilution method at 37±2°C is shown in Table 5. It showed the bacterial cell density in the soil samples fluctuated between 3.0×10^6 - 3.5×10^7 CFU/g, while in UEW samples it fluctuated between 1.0×10^6 - 5.3×10^7 CFU/ml. The average bacterial cell density was 1.34×10^7 CFU/g and 1.29×10^7 CFU/ml in the soil and UEW samples, respectively.

Percentage of HUB in TVC

The percentage of average of HUB in TVC was ranging from 18.5- 43.75% and 9.5 – 20.4% in the soil and UEW samples respectively. The average density of HUB relative to TVC was 27.6% and 15.8% in the soil and UEW samples respectively (Table 5).

Table. 5 Quantitative bacteriological analysis

S. No.	Sampling Site			HUB CFU/1ml UEW/1g Soil	TVC CFU/1ml UEW/1g Soil	% of HUB in TVC
1	Ankleshwar	CTF	Soil 1	4.5×10^6	0.23×10^8	19.57
2	Ankleshwar	CTF	UEW 1	2.0×10^6	0.12×10^8	16.67
3	Ankleshwar	GGS-W	Soil 2	3.0×10^6	0.16×10^8	18.75
4	Ankleshwar	GGS-W	UEW 2	1.0×10^6	0.105×10^8	9.52
5	Ankleshwar	Ank #W	Soil 3	5.0×10^6	0.27×10^8	18.52
6	AH 1	K#X	Soil 4	3.5×10^7	0.08×10^9	43.75
7	AH 1	K#Y	Soil 5	3.0×10^7	0.073×10^9	41.10
8	AH 1	GGS-X	UEW 3	5.3×10^7	0.26×10^9	20.38
9	AH 2	L#Z	Soil 6	3.0×10^6	0.126×10^8	23.81
10	AH 2	GGS-Y	UEW 4	5.0×10^6	0.254×10^8	19.69
11	AH 3	GGS-Z	UEW 5	3.25×10^6	0.252×10^8	12.90

Figure. 1 Quantification of DCPIP concentration at 600 nm absorbance (A) Isolate HUB1 (B) Isolate HUB2 (C) Isolate HUB3 (D) Isolate HUB4 (E) Isolate HUB5 (F) Isolate HUB6 (G) HUB Consortium
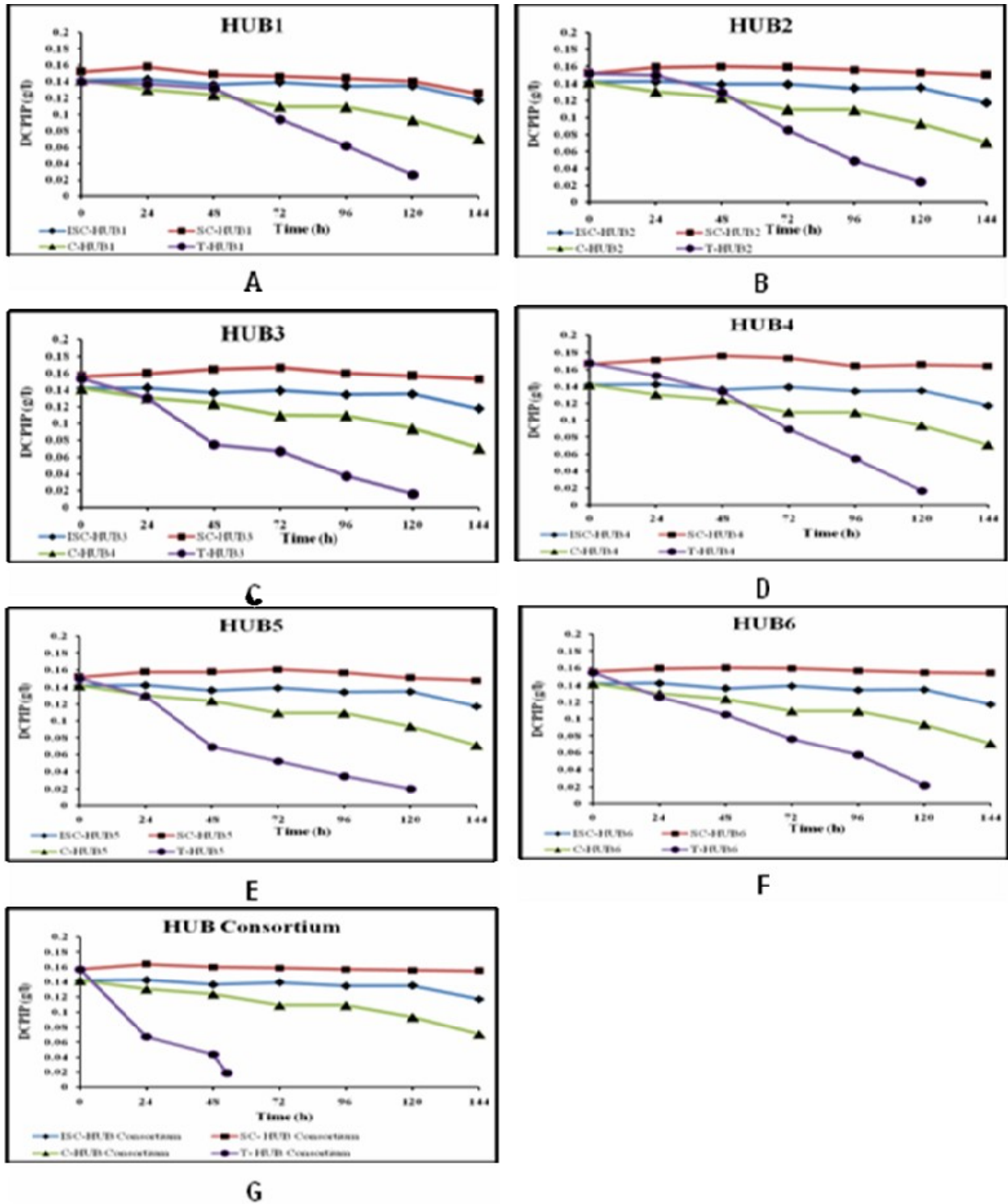
Enrichment and Isolation

Five UEW samples and six samples each of soil and crude oil were used for the enrichment and isolation of HUB. Sixty nine HUB isolates were obtained on BH medium as well as MS medium supplemented with 1% crude oil as sole source of carbon and energy. The isolates were then grown individually on MS medium and BH medium. In almost all cases, BH medium was found to be more suitable for the growth of these isolates, which was therefore used in subsequent studies.

Screening of hydrocarbon utilizing bacteria (crude oil from well K#X)

Following isolation and enrichment all the sixty nine isolates were screened for their efficiency of crude oil utilization / degradation (as a sole carbon source) using 2, 6- dichlorophenol indiphenol (DCPIP). Colour change of DCPIP was observed visually till 144 h. Based on the time for discolouration of DCPIP fifteen isolates were selected for further studies. Six isolates which decolorized DCPIP in the shortest time (about 120 h) were chosen for preparing the consortium. Both the isolates and consortium were further studied for decolourization of DCPIP spectrophotometrically by measuring absorbance at 600 nm, periodically, (Bidoia *et al*., 2010).

ISC-HUB consortium" and "SC-HUB consortium" did not show discolouration, whereas the substrate assays ("C-HUB consortium" and "T-HUB consortium") showed remarkable discolouration *i.e.,* oil was utilized. It was interesting to note that the "T-HUB consortium" assay completely decolourized DCPIP in 53 h. The biodegradation time observed for

consortium was less than half required by pure cultures of the isolates used for preparation of the consortium; however, less decrease was noticed in ISC-HUB$_n$ (from this point "$_n$" will be referred as 1, 2, 3, 4, 5, 6 and consortium) and SC-HUB$_n$ controls (Figures. 1A to 1G). DCPIP concentration below 0.030 g/l was considered as complete discolouration.

Microbial bioremediation as well as bioaugmentation are widely used techniques for treating hydrocarbon pollution in both terrestrial and aquatic ecosystems. Indigenous hydrocarbon degrading microorganisms play a significant role in this process. Research for standardizing *in situ* degradation process is required for a successful full-scale operation (Bidoia *et al*., 2010). The physico-chemical properties of oil-spill contaminated sites are essential for successful bioremediation process. These factors have direct influence on the type, number and metabolic activities of the microflora of any ecosystem (Adebusoye *et al*.,2008).

A strategy for screening of potential hydrocarbon utilizers / degraders involves sampling, isolation and screening of strains. Several methods of isolating and enumerating petroleum utilizing / degrading bacteria have been reported including cultivation on oil agar plates, silica gel oil media, and the Most Probable Number (MPN) method by inoculation of liquid media containing hydrocarbons (Afuwale and Modi, 2012).

Total sixty nine isolates were obtained from the various samples collected as described earlier. Eleven hydrocarbon degrading isolates from oil production site of Lingala oil field, ONGC, and nine isolates from oil contaminated soil from a

local area at Haridwar has been reported by Mittal and Singh (2009). Al-Thani *et al*., (2009) obtained a total of fifty one polyaromatic hydrocarbons (PAH) degrading isolates were reported from an industrial zone of Qatar, using naphthalene (20 isolates), phenanthrene (25 isolates) and anthracene (6 isolates) as the sole source of carbon (Al-Thani *et al*., 2009).

Bayoumi and Abul-Hamd (2010) reported isolation of one hundred and nine pure bacterial cultures was reported using MS medium supplemented with toluene and phenol as only source of carbon and energy (Bayoumi and Abul-Hamd, 2010). Afuwale and Modi (2012) have reported isolation of thirty six crude oil utilizing bacteria were isolated from the oil contaminated soil samples collected from ONGC field at Chandkheda (Afuwale and Modi, 2012).

Walker and Colwell (1976) stated that instead of total number of bacteria percentage of petroleum degraders in the total microbial population of the sample should be used if correlation was to be made between presence of petroleum degraders and concentration of oil in the sample. Joshi and Pandey (2011), have also indicated that the ratio of petroleum utilizing bacteria (PUB) to total aerobic heterotrophic bacterial count is more consistent and valid indicator than the absolute number of PUB in the sample.

Our studies showed a high bacterial count in all the oil contaminated samples. The average density of TVC in soil samples was 3.86×10^7 CFU/g, while in UEW samples was 6.66×10^7 CFU/ml, the latter count being higher. Youssef *et al.,* (2010) have reported a higher bacterial count in sediment than in the water sample(s).

The enumeration of HUB is an important criterion for the determination of potential for microbial degradation of oil-contaminated environments, and to assess the amount of oil pollution that has occurred (Walker and Colwell, 1976). The average density of HUB in soil samples was 1.34×10^7 CFU/g, while in UEW samples was 1.29×10^7 CFU/ml. The average density of HUB relative to the TVC in soil samples and UEW samples was 27.6% and 15.8%, respectively.

In this study results showed that average density of HUB relative to the TVC is higher in soil samples as compared to that in the UEW samples. The numbers of petroleum degrading microorganisms in water and sediment of Chesapeake Bay were related to concentration of oil present (Walker and Colwell, 1976). Singh and Lin (2008) demonstrated presence of diesel degrading organisms from diesel-contaminated soil samples from different transport companies in and around Durban, South Africa.

The Adebusoy *et al.,* (2008) carried out study with lagoon water sample from University of Lagos and reported the density of aerobic heterotrophic bacteria in the water sample was 2.7×10^5 CFU/ml, while the HUB was 1.91×10^4 CFU/ml. The density of hydrocarbon-utilizing species was 7.07% of the total heterotrophic population of the lagoon water ecosystem.. Joshi and Pandey have carried out studies on cow dung samples and reported the average count of total aerobic heterotrophic bacteria and PUB was 68.5×10^4 CFU/g and 11.5×10^4 CFU/g respectively. The relative density of PUB to total aerobic heterotrophic bacteria was found to be 17.47%. Hanson *et al.*, (1993) and Biodia *et al.,*(2010) have reported a number of techniques evolved for

screening of hydrocarbon degrading bacteria such as the use of liquid medium with hydrocarbons, oil containing mineral agar, measurement of turbidity in microtiter plates, O_2 consumption, MPN technique and colorimetric method.

Colorimetric methods are referred as cost-effective and rapid in detecting microbial metabolism, occurrence, both in aerobic or anaerobic instances. These catalytic microbial based methods are rapid, in which the natural co-substrate (which can be oxygen, sulfates or nitrates) is substituted by a synthetic mediator. Alternative means of determining the existence of reducing conditions include the use of redox indicators (Bidoia *et al*.,2010).

According to the results in "T-HUB Consortium" assay demonstrated more rapid biodegradation compared "C-HUB Consortium" assay. Even though no inoculum was added, biodegradation occurred in "C-HUB Consortium" assay probably due to indigenous microbial flora of the oil sample.

"ISC-HUB_n" assay indicates there is no reaction between BH medium ingredients and DCPIP. "SC –HUB_n" assay indicates DCPIP and BH medium together do not hamper growth of the organisms. Hence "ISC –HUB_n" and "SC –HUB_n" assays showed small or negligible decrease in DCPIP concentration which indicates an overall pattern of natural disintegration of DCPIP chemical structure through time. Therefore, it cannot be due to the inoculum added in the respective assay tubes. "C-HUB_n" and "T-HUB_n" assays revealed the decrease in DCPIP colour, which indicates a utilization of oil by bacteria. All "T-HUB_n" assays showed

rapid decrease in DCPIP absorbance as compared to "C-HUB_n" assays. "C-HUB_n" assays showed decrease in DCPIP colour due to the indigenous microbial population in crude oil sample used in this study.

Biodia *et al.,* (2010) reported that *B. subtilis* culture can completely reduce DCPIP at 138 h, 125 h, 75 h and 87 h for synthetic, semi-synthetic, mineral and used oil, respectively. Mariano *et al.,* (2009) compared the biodegradation of commercial and weathered diesel oils and concluded that the consortia had better biodegradation potential than pure cultures, as they may fail to exhibit degradation. The HUB isolates and consortium used in the present study have potentials for hydrocarbon utilization / degradation efficiently. Further studies on field trials using the consortium are envisaged.

Isolation and screening of microorganisms for their efficiency in utilization of hydrocarbons before field trials is important in bioremediation process. The development of efficient techniques is a cardinal tool when proposing different strategies for bioremediation of polluted areas for obtaining biodegradation data.

DCPIP decolourization method is rapid, easy and low cost for studying microbial utilization of hydrocarbons. Our initial studies has revealed that the bacterial consortium of HUB from various ONGC oil fields achieved crude oil degradation at less than half time (53 h) than that required by the pure cultures used for the purpose. Further, studies on optimization of the growth conditions as well as biodegradation capability for the consortia will provide an effective and eco-friendly technology for the degradation of hydrocarbons.

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