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Full Length Research Paper

Degradation of petroleum hydrocarbons by oil field isolated bacterial consortium

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A mixed consortium was prepared with 15 bacteria isolated by enrichment technique from the sample collected from an oil contaminated site. This consortium was incubated with crude oil to investigate the metabolic capability of bacteria. The degradation efficiency of the isolates in consortium was checked with 2% crude oil by shake flask transformation in mineral salt medium, at 37°C for 24 days. Total removal of aliphatic and aromatics was 94.64% and 93.75% respectively. Among the various components of the crude oil degradation by the bacterial consortium, the biotic removal of alkanes was maximum, 90.96% for tridecane (C_{13}) followed by pentadecane (C_{15}) at 77.95%, octadecane (C_{18}) at 74.1%, while other alkanes showed 56 to 69% after 24 days of incubation. The aromatics (benzene, toluene and xylene) were vaporized in the 4th day of incubation, while the efficiency on polyaromatic fractions (anthracene, phenanthrene and pyrene) was 46.17 to 55.3% after 24 days. The ability of degrading long chain n-alkanes and crude oil at high concentrations makes the consortium potentially useful for bioremediation and microbial enhanced oil recovery.

Key words: Consortium, petroleum hydrocarbons, crude oil, aliphatics, aromatics.

INTRODUCTION

Petroleum hydrocarbons can be introduced into the environment via oil spills, leaking or unplugged oil wells, the disposal ponds of waste petroleum products, abandoned oil refinery sites, pipe line ruptures, incomplete combustion of fossil fuels and accidental discharge during transport in tanks and ships failures. Petroleum production, drilling operations, and improperly sealed abandoned wells have caused major contamination of surface, ground waters and soils (US Environmental Protection Agency (USEPA), 1987; Richter and Kreitler, 1993; Kharaka et al., 1995; Kharaka and Hanor, 2003). Petroleum hydrocarbons can seep into soil and contaminate underlying ground water. Runoff from unregulated sites can carry petroleum contaminants off site into nearby waterways. Dumping of waste petroleum products, for example, lead to an elevated loading of petroleum hydrocarbons in soil, which results in significant decline in the quality of soil and makes it unfit for use (Shabir et al., 2007).

Remediation of petroleum contaminated sites could be achieved by either physicochemical or biological methods. Due to negative consequences of the physicochemical approach, more attention is now given to the exploitation of biological alternatives (Okoh, 2006). The persistence of petroleum pollution depends on the quantity and quality of the hydrocarbon mixture and on the properties of the affected ecosystem. Petroleum hydrocarbons may be completely biodegraded within a few hours or days depending upon condition (Atlas and Bartha, 1992). Most of the petroleum compounds are biodegradable but the process is very slow (Atlas and Bartha, 2001).

The most important principle of bioremediation is that microorganisms can be used to destroy hazardous contaminants or transform them to less harmful forms (US National Research Council, 1993). Since ZoBell (1946) reported that nearly 100 species of bacteria, representing 30 microbial genera, had hydrocarbon oxidizing properties, many species and genera have been found to have this ability (Texas Research institute, 1982a). The heterotrophic microorganisms found in the

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soil include naturally occurring populations that have the ability to degrade petroleum products (Englert et al., 1993). *Pseudomonas*, *Arthrobacter*, *Alcaligenes*, *Corynebacterium*, *Flavobacterium*, Achromabacter, Micrococcus, Nocardia, and Mycobacterium appear to be the most consistently isolated hydrocarbon degrading bacteria from soil (Englert et al., 1993).

There is no single strain of bacteria with the metabolic capacity to degrade all the components found within crude oil. In nature, biodegradation of a crude oil typically involves a succession of species within the consortia of microbes present. A combination of bacterial strain with broad enzymatic capabilities is required for active extensive degradation of crude oil. Oil degrading mixed cultures can be constructed by combining a number of strains with known degradative capabilities (Fought et al., 1999; Komukai et al., 1996). Enrichment technique can also be utilized to isolate mixtures of oil degrading bacteria from contaminated sites and make the consortia (Budzitoki et al., 1995; Sugiura et al., 1997).

This paper describes the formation of bacterial consortium by isolates from oil contaminated sites, having the ability to utilize crude oil as growth substrate. The consortium was subjected to metabolic characterization. It was incubated with crude oil to study the degradation of complex hydrocarbons. Mineral salt medium with oil was used as sole carbon source.

MATERIALS AND METHODS

Microorganisms and cultural conditions

For the routine, a cultured bacteria nutrient agar was used. For the biodegradation experiment mineral salt medium (PNR) as described by De Frank and Ribbon (1976) with crude oil taken from the local oil field was used as sole carbon and energy source as emulsion (2 to 5%) was used. The oil sample was kindly provided by OGDCL Pakistan. The medium was sterilized before the addition of oil. The oil was sterilized at 121°C for 20 min in closed vials.

Identification of selected bacterial isolates

The identification of bacterial isolate with the ability to degrade crude oil was performed on the basis of microscopic examination and biochemical tests according to Bergey's manual of determinative bacteriology (Holt, 1993). The bacterial isolate was identified according to colonial morphology, surface, shape, size, margin and pigmentation on nutrient agar medium. The microscopic examination included gram staining, capsule staining, and spore staining. The motility test was also performed. The biochemical tests including citrate utilization, oxidase production, starch hydrolysis, lipid hydrolysis, casein hydrolysis, methyl red, voges proskauer test (MR-VP), nitrate reduction test, gelatin liquefaction test, triple sugar iron test (TSI) for lactose, dextrose, sucrose, glucose and manitol fermentation, carbohydrate fermentation, H_2S production, indole production test and urease test were carried out according to standard procedures.API (50 CH BIOMERIEUX, France) kit was also used to confirm the biochemical characterization of isolated bacterial strains.

Biodegradation studies

Biodegradation of crude oil by bacterial consortia

The biodegradation assay was carried out in 250 ml Erlenmeyer flasks containing 100 ml of mineral medium and 2% of crude oil as the sole source of carbon and energy. The inoculum (10% v/v) contained cells from enrichment cultures, sub cultured in mineral broth (over night cultures) and mixing thoroughly. The oil cultures were incubated for 3 weeks at 37°C temperature with agitation of 130 rpm on a rotary shaker in an incubator. Growth was monitored by testing optical density (OD) at 620 nm after every 4th day. The collected samples were extracted and analyzed to check the total petroleum hydrocarbon degradation by gas chromatography. Non inoculated flasks were included as control for abiotic losses. Assay was carried out in duplicates.

Analysis of crude oil samples

Samples (2 ml) were taken from flask with disposable pipette. The flasks were shaken vigorously to suspend the solid materials in order to obtain homogenous sample. The samples were extracted ultrasonically with mixture of methanol, di-chloromethane (DCM) and water (1:5:4), centrifuged for 15 min. The extracts were dried over with $NaSO_4$ and concentrated in rotary evaporator to final volume of 5 ml. A 1 ml aliquot was separated and was diluted to concentration appropriate for Gas chromatography (GC).

Gas chromatography

Crude oil components were separated on a capillary column DB1 (Manufacturer J & W) length of 30 m. Then nitrogen was used as carrier gas at pressure of 2.0 kg/cm² measured at injector 20 ml par min with total flow of 33.6 ml per min. The hydrogen flow, 35 ml par min and air flow of 350 ml par min during running process. The initial column oven temperature was 35°C (for 1 to 5 min).The ramp 1 was 5 to 10°C par min to 300°C (for 20 min) and ramp 2 was 4 to 10°C par min to 280°C. This final temperature was held for 20 min with maximum temperature exceeding 350°C. The total run time was 74 min.

Individual components, aliphatic and aromatic hydrocarbons, were used as standards (standard match method). A commercial standard mixture ASTM (American Society for Testing Materials) 110, D2887 calibration mixture was also used to calibrate the GC column.

The concentrations of the petroleum components were calculated from the peak area chromatograms then total aliphatic components and aromatic components were calculated to compare the percentage degradation at different time and intervals of the biodegradation experiment. The effects of different percentage on individual components were also analyzed in specific cases. The results presented are the average of duplicate experiments.

RESULTS

Isolation and characterization of crude oil degrading strains

15 bacterial isolates were selected on the basis of their growth on crude oil containing mineral salt medium, characterized on the basis of morphological and biochemical characteristics according to Bergey's Manuals

Table 1. Removal of crude oil (2%) degradation by bacterial consortium.

Oil Composition	Concentration of oil components in parts per million (ppm)							Percent removal	Biotic removal (%)
	0 time	4th day	8th day	12th day	16th day	20th day	24th day		
Aliphatic hydrocarbons conc. (ppm)									
C_8	3839.142	0	0	0	0	0	0	100	0
C_9	4527.771	353.102	325.651	0	0	0	0	100	0
C_{10}	3628.991	1159.49	1026.451	0	0	0	0	100	0
C_{11}	5989.412	1634.77	1299.541	126.355	54.079	0	67.724	98.86	2.43
C_{12}	6725.148	4482.63	4161.542	987.945	314.9	861.998	174.14	97.41	8.18
C_{13}	7864.381	6322.24	5391.412	1787.36	554.774	1125.757	232.645	97.04	90.96
C_{14}	7599.205	7058.59	8093.451	3439.73	693.447	1816.488	1242.83	83.64	57.96
C_{15}	5633.841	2826.07	2745.654	2242.04	1154.414	649.742	438.427	92.21	77.98
C_{16}	7288.691	5514.82	4413.216	3832.64	1160.114	1142.932	639.383	91.22	64.44
C_{17}	7866.601	4858.81	3654.025	3640.99	1610.13	871.229	721.25	90.83	69.38
C_{18}	6005.448	2259.16	2189.365	3067.5	1181.135	238.873	378.71	93.69	74.1
C_{21}	3074.412	2167.19	2541.321	3262.28	1056.852	240.628	167.272	94.55	69.93
C_{24}	2827.369	1021.93	1002.963	1477.8	512.874	70.366	0	100	56.01
C_{28}	2055.124	283.914	264.321	687.984	322.345	0	0	100	58.19
C_{32}	994.657	126.158	129.549	173.842	190.704	0	0	100	68.7
Total:	75920.19	40068.85	37238.46	24726.46	8805.768	7018	4062.378		
%Deg:	0	47.22	50.95	67.43	88.40	90.75	94.64		
Aromatic hydrocarbons conc. (ppm)									
Benz	3545.661	0	0	0	0	0	0	100	0
Toluene	7236.102	0	0	0	0	0	0	100	0
Xylene	4076.551	1367	0	0	0	0	0	100	0
Naphth	3406.881	3076.49	2691.324	317.043	90.451	303.52	234.861	93.10	16.19
Ant/pha	7736.841	7606.07	6854.321	4479.33	1630.291	1428.091	909.211	88.24	55.3
Pyrene	3641.872	1978.99	1786.541	2547.79	1176.106	189.357	128.403	96.47	46.17
Total:	29643.9	14028.55	11332.19	7344.163	2896.848	1920.968	1272.475	95.70	
%Deg:	0	52.67	61.77	75.22	90.31	93.51	95.70		

of Determinative Bacteriology (9th Edition), and Manual for Identification of Medical Bacteria (3rd Edition) by Cowans and Steel (1993). 7 strains were Gram positive rods (MD-1, DW-3, SS-1, SNW-3, SNW3-2, SNW3-3 and SNW4-4) identified as *Bacillus* sp, 3 (DW-1, DW-8 and SNW3-1) were cocci belonging to *Micrococcus* sp. and *Staphylococcus* sp., and 3 strains (DW-6, SNW3-4, SS1-8, SS-2 and SOL-10) were Gram negative small rods identified as *Pseudomonas*, *Alcaligens* and *Psychrobacter* sp.

Degradation by bacterial consortium

The experiment was performed to study the biodegradation of crude oil by consortium of 15 bacterial isolates. Inocula in nutrient broth was prepared to increase cell biomass and then mixed and used as consortium. The cultures that were incubated for 24 days showed high degree of degradation more than those (cultures) at 4, 8 12, 16, and 20 days.

GC analysis of total extract showed that the consortium caused a complete depletion of the major components. If taken, total aliphatic and aromatic components degradation was 94.84 and 93.75% respectively after 24 days of incubation. Degradation of target compounds, running as standards, were analyzed and found that lower alkanes C8, C9 and C10 were depleted almost 100% in 12 days (Table 1). These fractions were also removed without inoculum, indicating abotic removal by physical factors. When degradation was assayed and measured as removal by biotic fractions by subtracting the percentage removal in the test (with inoculum) from the control (without inoculum) different rates of removal of n-alkanes were observed.

The GC chromatograms for aromatic compounds demonstrated that the consortium caused considerable degradation of the compounds containing two, three and four aromatic rings. The results of the quantification of selected compounds are shown in Table 1. Mono aromatics were removed by vaporization, and 100% removal was observed both in test and control samples

within 4 days of incubation.

Among the poly aromatic hydrocarbons, anthracene and pyrene depleted to 55.3 and 46.17% respectively after the 24th day, with bacterial consortium (Figure 3). When the time course for degradation of individual components of aliphatics was followed, major degradation takes place between 0 to 4 days (Figure 1). The pattern was also same but the removal was progressive and slow except for C11, C12 and lower alkanes C8 and C9 removed by abiotic factors in initial 4 days (Figure 2).

The differences in the chromatograms were most noticeable between 0 and the 4th day of the incubation where the majority of *n*-alkanes below C11 and the aromatic had been degraded. Between the 8th and the 16th day, most of the C12 to C18 components were removed. Between day 16 and day 24 of the incubation period, the amount of degradation of high molecular weight compounds greater then C21 was evident along with polyaromatic hydrocarbons (Figure 1). When the bacteria growth was checked during the experiment, there was gradual increase till the 20th day and then further decrease so the degradation follows the growth of bacteria (Figure 4).

DISCUSSION

The degradation of crude oil by bacterial strain isolated from oil contaminated drilling mud by selective enrichment techniques; the microorganisms implicated in oil degradation are widely distributed in nature and have been isolated from soil and water ecosystems with their oil degrading potentials (Bello, 2007). The microorganisms capable of utilizing oil and oil products as a sole source of carbon and energy occur practically everywhere in air, water and soil (Olliver and Magot, 2005). It is estimated that in 1 g of unpolluted soil, there are only 100 to 1,000 cells of hydrocarbon degrading microorganisms, whereas, in 1 g of soil polluted by oil, their number increases to 1×10^6 to 5×0^7 cells, especially if pollution occurred repeatedly and during a long time (Rosenberg and Ron 1996).

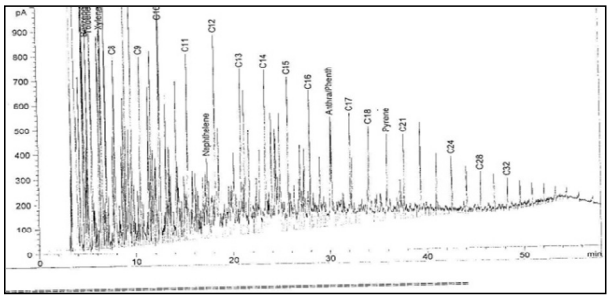
Taxonomic charecteristics of these isolates identified them as *Bacillus* sp, *Staphylococcus* sp, *Micrococcus* sp*, Pseudomonas* sp*, Psychrobacter* sp, and *Alcaligens faecalis*. There are other reports of such oraganisms also reported before (Sathishkumar et al., 2008; Lal and Khanna, 1996; Rahman et al., 2002). *Micrococcus*, *Staphylococcus*, *Pseudomonas putida* and *Alcaligene*s were also reported to degrade diesel oil (Pepi et al., 2003).

The pattern of degradation showed that the microorganisms first attacked the lower and higher hydrocarbon chains and those of middle length were attacked later in the course of incubation (Bello, 2007). Considerable information on the microbial degradation as defined, sole hydrocarbons is available in the literature, but less is known on the biodegradability of some petroleum commercial products (Sugiura et al., 1997) such as kerosene (Wongsa et al., 2004). The dominant mechanism that breaks down these petroleum products is biodegradation, which is carried out by natural microbial populations (Kokub et al., 1990; Margesin and Schinner, 2001a). Crude oil, as well as other commercial hydrocarbons, can be considered extensively biodegradable in soils, but with differences in the extent of hydrocarbon degradation (Morgan and Watkinson, 1989; Leahy and Colwell, 1990; Bossert and Compeau, 1995; Huesmann, 1997).

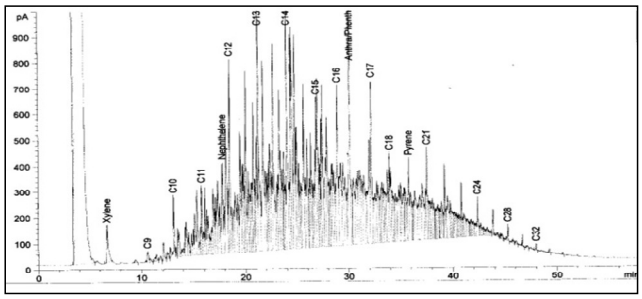
In the present study, 2% crude oil degradation was studied in liquid medium by consortium of 15 different bacterial strains. The result shows that the degradation of n-alkanes up to C_{16} occurred simultaneously best at different rates. For *n*-C_{28} and *n*-C_{32}, there is a long delaying before degradation even started to occur (between 16 to 24 days). This may indicate that as the amount of lower molecular weight n-alkanes dramatically decreased, the bacteria were forced to utilize the higher molecular weight in alkanes and this may require some kind of enzymatic modification. Alternatively, another explanation for the delay would be the concentration effect that is when the lower molecular weights alkanes were present; the hydrocarbon degrading bacteria may not degrade the molecular weight compounds until the low molecular compounds were almost degraded (Heath et al., 1997).

The consortium affectivity biodegraded the crude oil in liquid culture, Stimulated biodegradation of crude oil is at present being encouraged because it ensures rapid remediation of oil polluted ecosystems (Ijah and Antai, 2003). Earlier studies have shown that a bacterial consortium comprising hydrocarbon degrading *Pseudomonas aeruginosa* (1 strain) and *Bacillus* sp (2 different strains), it could effectively biodegrade crude oil petroleum in liquid cultures as well as in polluted soil and sand (Salleh et al*.,* 2003). The addition of bacterial consortium and nutrients resulted in an effective bioremediation response (Bourquin, 1996; Limbert and Betts, 1996). Several species of oil degrading fungi and bacteria have been detected in the sand soils of the Arabian Gulf, which need to be examined for their role in the natural biodegradation of petroleum in contaminated soils (Hashem, 1996; Radwan, 1991). The large diversity of molecules contained in petroleum products favors the co-oxidation phenomenon. Therefore, petroleum products are degraded in the environment by mixed populations, but studies on the utilization in soil of complex hydrocarbon mixtures by bacterial consortia are few (Alvarez and Vogel, 1991; Arvin et al., 198; Song et al., 1990). In one case all members of the consortium belonged to the same genus and they probably carried the same hydrocarbon degrading gene(s). The composition of a microbial consortium is an important factor, which must be made to ensure synergistic enhancement of catabolic

Zero time



Day 4



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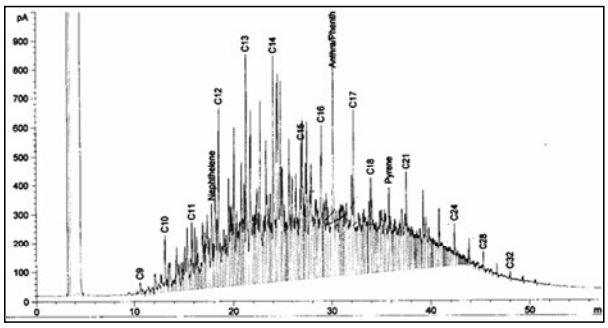

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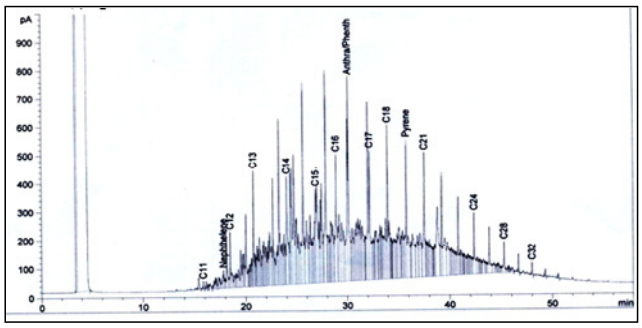
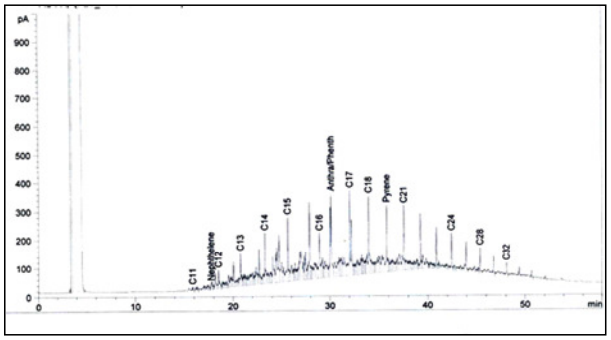
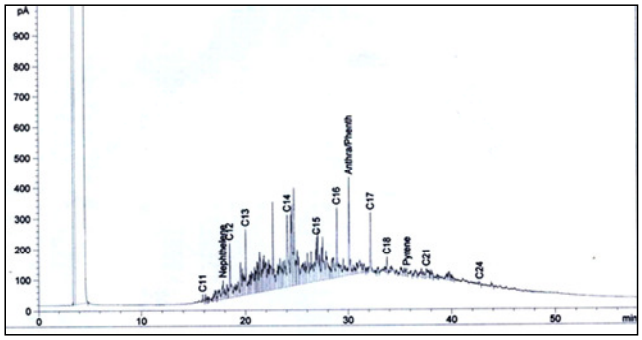


Day 16



Day 20



Day 24

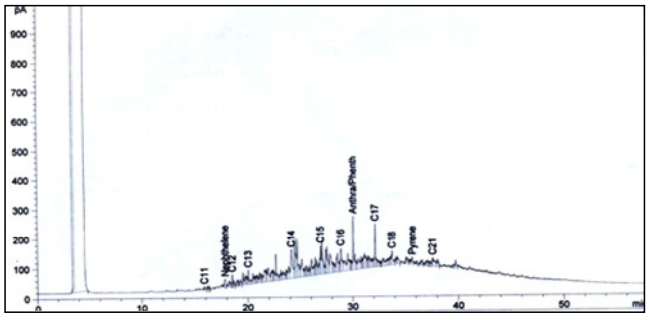


Figure 1. Gas chromatograms of aliphatic and aromatic fractions of crude oil at selected intervals during 24 days degradation experiment with bacterial consortium.

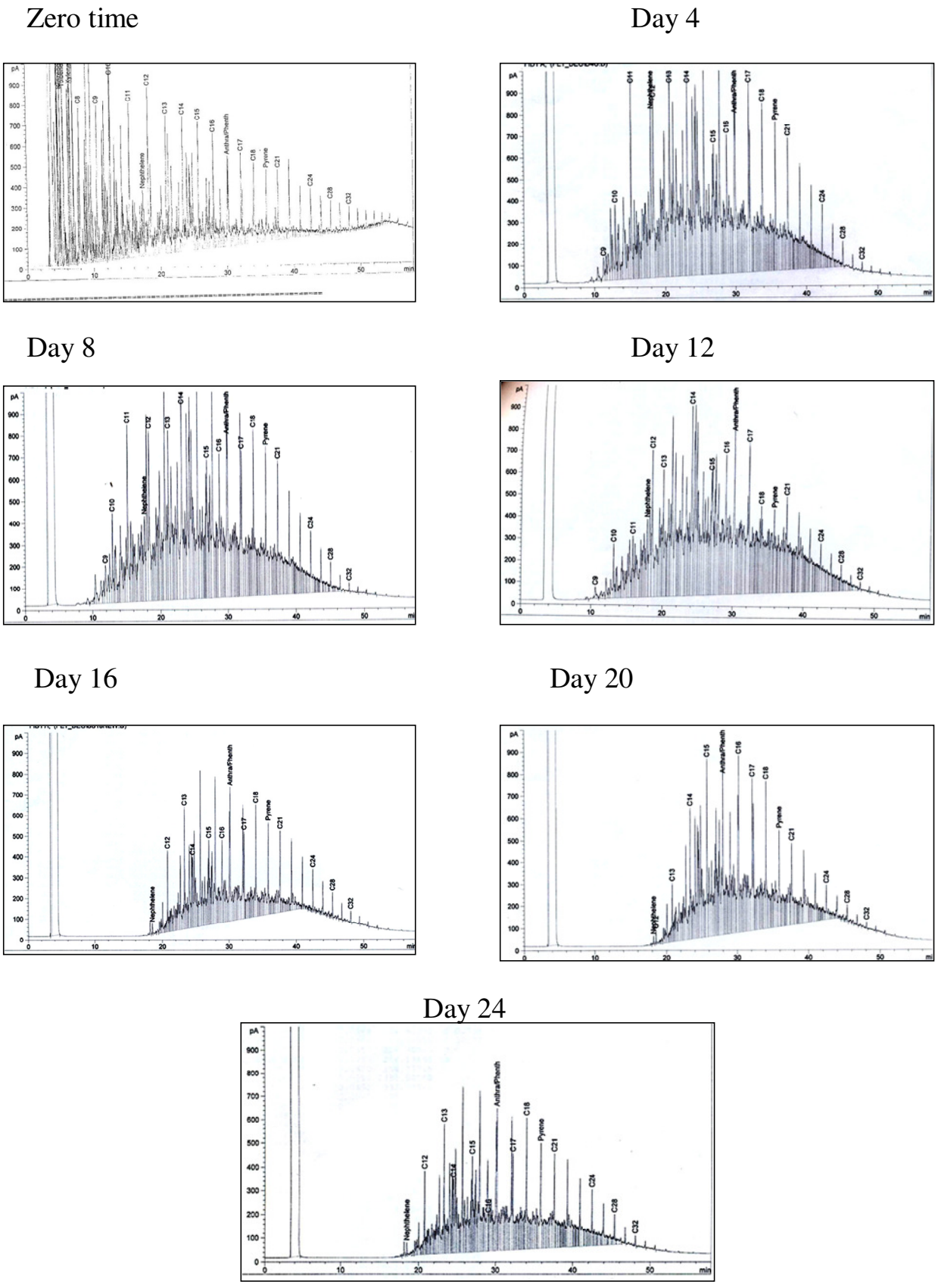


Figure 2. Gas chromatograms of aliphatic and aromatic fractions of crude oil at selected intervals during 24 days degradation experiment in control without inoculum.

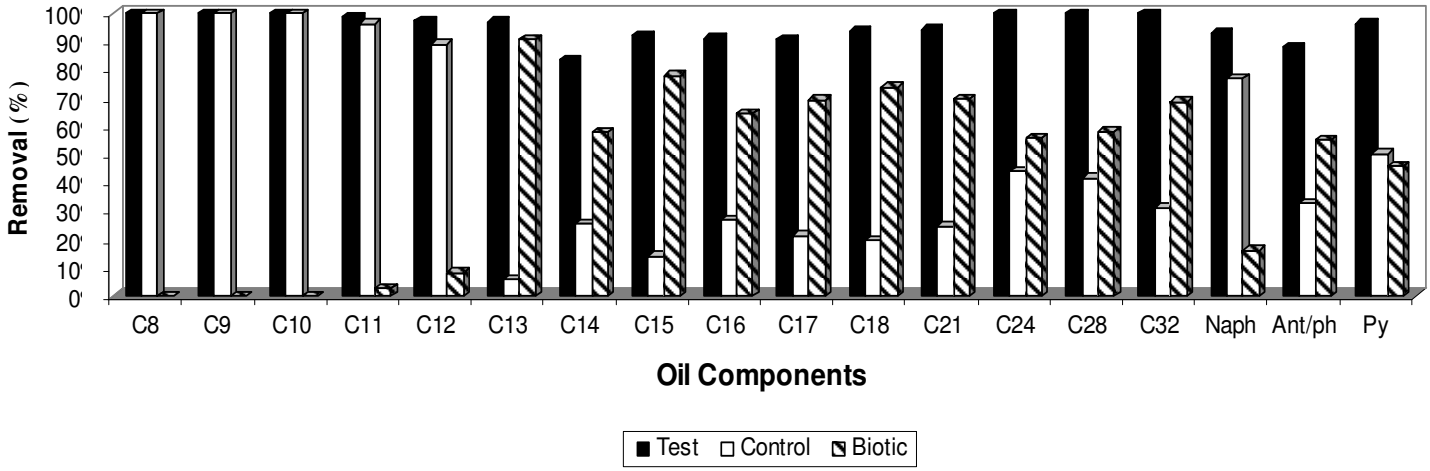


Figure 3. Percent removal of 2% crude oil components after 24 days test (bacterial consortium) and in control (abiotic removal).

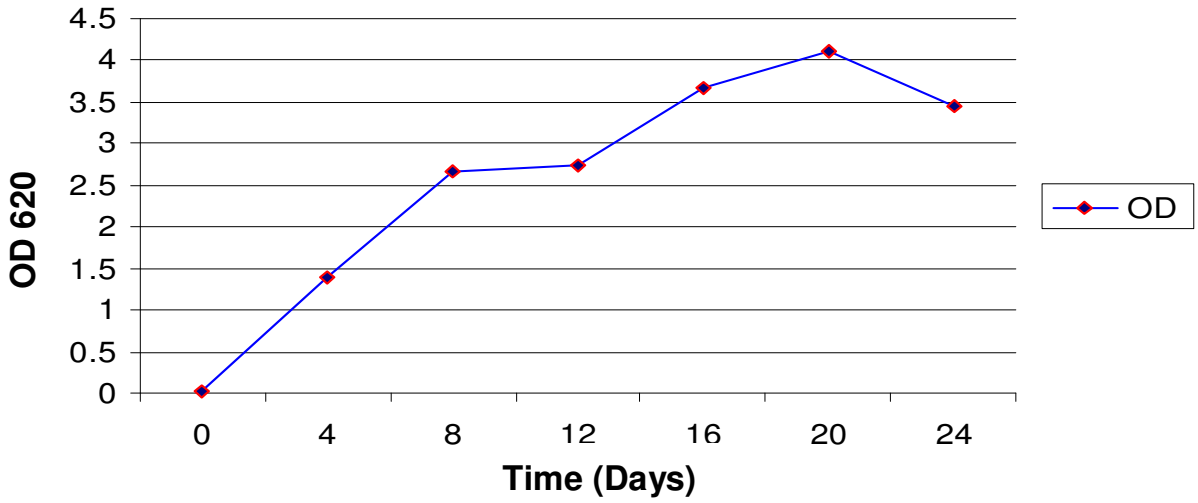


Figure 4. Growth optical density of bacterial consortium in 2% oil degradation

activities, it was reported that a microbial consortium exhibited higher activity than an axenic culture of *Acinetobacter* for the biodegradation of light and heavy crude oils (Haramaya et al., 1997). Total removal of the n-alkanes was 90.34% and the aromatic compounds were as 93.75% in 24 days. The lower alkanes C_8-C_{10} were removed by vaporization within 4 to 8 days. Removal by biotic factors was calculated by subtracting the percentage removed in the test flask from that removed in the control flask, the higher percentage biotic removal for the C_{13}-C_{18} and the higher alkanes (C_{21}, C_{24} and C_{28}) was 56 to 60% in 24 days while the middle range compounds had a somewhat lower level of degradation (C_{16} 51% and C_{17} 40%). Among the aromatics the micro aromatics quickly evaporated and after the 4th day no peak was detected. While the fully aromatics hydrocarbons of 3 and 4 ring compounds showed 51 to 68% biodegradation (Figure 1). Biodegradation or utilization of oil by the bacterial consortium resulted in the growth of bacteria, as there was a gradual increase in OD (620) until day 20 after the further reduced (Figure 4). The degradation was also progressive with during the maximum degradation occurring between 12 to 16 days (Table 1). Abiotic removal was maximized during the initial 4 to 8 days (Table 2).

Lal and Khanna (1996) reported 58% overall degradation of Indian crude oil samples by *Acinetobacter calcoaceticus* and *Alcaligen odorans* in combination over a 15 day period. Ijah (1998) reported that bacteria and yeast isolates from tropical soils capable of degrading 52% and 69% of crude oil in 16 days, respectively, where;

Table 2. Removal of crude Oil (2%) by abiotic factors (control).

Oil Composition	Concentration of oil components in parts per million (ppm)							Removal percentage
	0 time	4th day	8th day	12th day	16th day	20th day	24th day	
Aliphatic hydrocarbons conc. (ppm)								
C_8	3839.142	0	0	0	0	0	0	100
C_9	4527.771	1205.551	0	0	0	0	0	100
C_{10}	3628.991	2961.227	0	0	0	0	0	100
C_{11}	5989.412	4007.904	195.663	165.357	1722.456	1236.337	933.551	96.43
C_{12}	6725.148	4921.307	1255.228	907.224	4225.321	4215.994	2211.504	89.23
C_{13}	7864.381	5677.159	3221.502	3055.109	4051.661	6094.441	1866.541	6.08
C_{14}	7599.205	6011.457	5661.114	5088.254	5224.124	5988.412	4691.503	25.68
C_{15}	5633.841	3651.781	7706.551	7523.448	2661.188	2288.664	1699.661	14.23
C_{16}	7288.691	6691.101	4236.224	3966.215	4041.557	4435.218	3077.447	26.78
C_{17}	7866.601	7702.658	7001.664	6881.012	6601.852	7500.664	5588.509	21.45
C_{18}	6005.448	5022.141	5966.106	5066.318	3599.547	3998.774	3788.124	19.59
C_{21}	3074.412	2055.918	4550.374	4022.226	1652.441	1702.558	1187.361	24.62
C_{24}	2827.369	1905.663	2991.204	2066.227	1109.514	2051.667	1774.105	43.99
C_{28}	2055.124	1102.654	1532.228	1406.334	771.529	778.641	473.551	41.81
C_{32}	994.657	701.542	409.551	499.203	305.901	399.124	280.904	31.30
Total:	75920.19	53618.06	44617.41	40481.57	38020.97	41703.38	28462.79	0
%Deg:	0	29.37	39.70	45.29	49.91	59.07	62.50	0
Aromatic hydrocarbons conc. (ppm)								
Benz	3545.661	0	0	0	0	0	0	100
Toluene	7236.102	0	0	0	0	0	0	100
Xylene	4076.551	0	0	0	0	0	0	100
Naph	3406.881	3201.447	2731.251	2607.115	2896.304	1324.785	786.551	76.91
Ant/pha	7736.841	7906.428	7054.405	6419.552	6179.206	5833.417	5188.305	32.94
Pyrene	3641.872	2864.661	1866.741	2297.119	2507.431	1830.664	1809.667	50.30
Total:	29643.9	13972.54	11652.4	11323.8	11582.9	8988.87	7784.52	
% Deg:	0	52.86	60.69	61.80	60.92	69.67	73.73	

the isolates primarily degraded the alkanes over specific carbon number ranges.

The alkanes *n*-C_{24} to *n*-C_{28} was degraded about 40 to 45% within 24 days at 37°C in liquid culture while C_8 to C_{12} were removed at 89 to 100%. The middle range compounds of *n*C_{14}-*n*C_{21} showed 20 to 30% degradation. The distribution of recovered alkanes suggested a preferential degradation of shorter chain molecules (*n*-C_8 to *n*-C_{12}) by the bacterial consortium (Ambrosch et al., 2007). According to Atlas (1981), there are many kinds of bacteria capable of degrading these compounds (Whyte et al., 2002). So when removal of individual hydrocarbons was also tested in the control flask without the inoculum, the removal of lower alkanes and aromatic was observe up to 100% for some of the compounds. Therefore, the disappearance of significant quantities of short chain n-alkanes (C_8-C_{11}) during the experiment and the decrease of total hydrocarbons (aliphatic and aromatics) could also be related to abiotic factors, such as evaporation and dispersion as explained by Luzi et al. (2006). Mixed aqueous cultures of hydrocarbon degrading bacteria also show a first order degradation pattern. Dott et al. (1989) reported degradation percentages (1% fuel oil) from 24 to 64% from commercially available inocula for oil degradation. The degradation of n- alkanes in these cultures was from 50 to 90% removal of aliphatic and aromatic components.

In a similar study, Sugiura et al. (1997) reported 19 to 34% degradation of total extracts of different crude oils by non-defined mixed culture obtained by enrichment in artificially weathered crude oil, but the aliphatic fraction was only removed by 40 to 53%, Pristane and phytane were removed upto 75 to 100% depending on the crude oil. Pallitopongarnpim et al. (1998) reported 26 to 63% biodegradation of Tapis crude oil TPHs by three different single bacterial isolates, however, these figures were based on depletion of the resolved peaks in GC chromatographic profiles. In fact, the chromatograms showing degradation of the total extract of Topis crude oil by the best oil degrader (*C. tropicalis* MU15Y) show the persistence of some major peaks. Chhatre et al. (1996) reported a 70% degradation of the major peaks resolved

in GC of Bombay high crude oil by a defined consortium constructed with four oil degrading bacterial strains for their capability to degrade saturates and aromatics.

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