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Degradation of 2T (2-stroke oil) Engine Oil by *Acinetobacte*r sp. Isolated from Automobile Workshop Contaminated Soil of Kurukshetra

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Biodegradation of hydrocarbon-contaminated soils has been established as an efficient, economic, versatile and environmentally sound treatment. A total of nineteen morphologically different bacterial cultures were isolated. The growth of isolated cultures was studied in liquid M9 medium containing 0.5 % 2T engine oil. On the basis of increased OD in M9 medium, bacterial strains GD2 was selected for further study. On the basis of morphological, physiological and biochemical properties strain GD2 was tentatively identified as Acinetobacter sp. It was found that the GD2 strain degraded the following hydrocarbons (Trans, trans-1-6-dimethylspiro [4, 5] decane, Cis trans-1-6-dimethyl spiro [4, 5] decane, Pyrrolo [1, 2-a] pyrazine-1, 4-dione, hexahydro-3-(2-methylpropyl), Octateiacontyl pentafluoropropionate, Octacosyltri fluoroacetate, Triacontylacetate, Tetra triacontyl pentafluropropionate, Tetra triacontyl trifluoroacetate and Tetradecane, 1 bromo) present in 2T engine oil. Capability to degrade hydrocarbons makes it potential organism for consideration in the bioremediation of by 2T engine oil contaminated sites.

Key words: 2T engine oil, *Acinetobacte*r sp, Biodegradation, Enrichment, Hydrocarbon.

Lubricating oil consists of about 5-20% (w/w) chemical additives in a base fluid which is a complex mixture of hydrocarbons including linear and branched paraffins, cyclic alkanes and aromatic hydrocarbons (Jirasripongpun 2002). This engine oil renders the environment ugly and constitutes a potential threat to humans, animals and vegetation (Edewor *et al.,* 2004). Fat soluble components may accumulate in the organs of animals and may be enriched in the food chain, even up to human (Mackay and Fraser 2003). Prolonged exposure and high oil concentration may cause the development of liver or kidney disease, possible damage to the bone marrow and an increased risk of cancer (Mishra *et al .,* 2001). Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution (Holliger *et al.,* 1997). The accumulation of pollutants in animals and plant tissue may cause death or mutations (Alvarez and Vogel 1991). In contrast, to the physicochemical treatment methods for removal of oil, such as; incineration and solvent extraction, biodegradation offers a cost effective and environment friendly technology (Vasudevan and Rajaram 2001). The potential for biodegradation of oil by microbes is well established (Mohanty and Mukherji 2008). These microorganisms can degrade a wide range of target constituents present in oily sludge (Barathi and Vasudevan 2001). Bioremediation processes have been shown to be effective methods that stimulate the biodegradation of contaminated soil and may restore contaminated

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soils through the broad biodegradative capabilities evolved by microorganisms towards undesirable organic compounds (Andreoni and Gianfreda 2007). Bacterial enrichment, isolation and crude oil degradation in aqueous medium was observed (Adebusoye *et al.,* 2007). The biodegradation of oil pollutants has been intensively studied in controlled condition and in open field experiments (Chaillan *et al.,* 2006). In the present study, enrichment and isolation of 2T engine oil degrading baterial cultures was performed from automobile workshop contaminated soil. The main objective was to study biodegradability of 2T engine oil by using enriched culture.

MATERIALS AND METHODS

Soil sample and Chemicals

The oil contaminated soil samples were collected from automobile workshops of kurukshetra. The chemicals, media and reagents used in the present studies were purchased from Hi Media Laboratories, CDH and Rankem etc. The chemicals were of AR grade. Analytical and spectroscopic grade hexane and acetone was purchased from CDH & Rankem respectively. All other chemicals were available of AR grade commercially.

Isolation, identification, growth and antibiotic resistance pattern of bacterial culture utilize 2T engine oil as a sole carbon source

A defined M9 medium was used for the isolation of 2T engine oil degrading bacteria[1]. Approximately 10 g of soil sample was suspended in 100 ml M9 medium supplemented with 0.5 % 2T engine oil and incubated at 37 ^{0}C. After 7 days of incubation at 30 ^{0}C, 10 ml enriched sample was transfer to 90 ml fresh M9 medium containing 0.5 % 2T engine oil. Subsequently four rounds of enrichment were carried out. Streaking was done from enriched medium on nutrient agar plates for the isolation of bacterial cultures. Isolated colonies were transfer to nutrient agar slants for further study. The identification was carried out by using Hi-media biochemical test kit. The growth was observed by inoculated 500 µl strain GD_2 inoculum into 10 ml M9 medium containing 100 µl 2T engine oil. The uninoculated medium act as control was also incubated. The optical density was measured after 0, 3, 6, 9, 12 days at 600 nm on spectrophotometer. Identification of strain GD_2 was carried out by using Hi-media biochemical test kit. The antibiotic resistance pattern was determined by disc diffusion method.

Quantitative analysis of 2T engine oil degradation by GC-MS

The degradation potential was checked by GC-MS analysis (Nguyen 1999). The strain GD_2 was grown in 300 ml nutrient broth supplemented with 0.5 % (v/v) 2T engine oil at 37 ^{0}C up to mid log phase. Cells were harvested at 4 ^{0}C and washed twice with M9 medium. These cells were resuspended in 50 ml M9 medium containing 0.5 % (v/v) 2T engine oil. Aliquots of 10 ml were taken at different time interval of 0, 5 and 10 days. The 2T engine oil from cell free supernatant was extracted by using 40 ml hexane: dichloromethane (1:1) for 30 min. The degradation of hydrocarbons present in 2T engine oil was determined by GC-MS.

RESULTS AND DISCUSSION

Isolation, identification and antibiotic resistance pattern of bacterial culture GD_2

Hydrocarbon-degrading bacteria and fungi are widely distributed in marine, freshwater, and soil habitats. The recognition of biodegraded petroleum-derived aromatic hydrocarbons in marine sediments was reported by (Jones *et al.,* 1983). In the present study, enrichment method was used for the isolation of 2T engine oil degrading bacteria from automobile workshop contaminated soil. A total of nineteen morphologically different bacterial cultures were isolated by using enrichment method. The growth pattern of all nineteen isolated bacterial cultures were checked in liquid M9 medium containing 0.5 % 2T engine oil at 600 nm. On the basis of increasing OD in M9 medium containing 0.5 % 2T engine oil, strain GD_2 was selected for further study. The strain GD_2 was utilizing 2T engine oil as a sole source of carbon and energy. The absorbance of bacterial strain GD_2 at different time interval was monitored as (0.02, 0.20, 0.40, 0.52 and 0.51) at 600 nm respectively. The strain was Gram's negative with rod shape cells. On the basis of morphological, physiological and biochemical properties bacterial strain GD_2 was closely related to *Acinetobacter sp.* GD_2 strain showed growth at (30 ^{0}C, 37 ^{0}C and 42 ^{0}C) but was unable to grow at (4, 10, 15 and 20 ^{0}C,

Table 1. Hydrocarbons degradation of 2T engine oil by GD_2 strain.

Name of hydrocarbons	5 day	10 day
Trans, trans-1-6-dimethylspiro [4, 5] decane	24.14 %	100 %
Cis trans -1-6-dimethylspiro [4, 5] decane	28.57 %	100 %
Pyrrolo[1, 2-a] pyrazine-1, 4-dione, hexahydro-3-(2-methyl propyl)	27.78 %	100 %
Octateiacontylpentafluoropropionate	43.81 %	59.23 %
Octacosyltrifluoro acetate	37.77 %	64.10 %
Triacontyl acetate	48.20 %	100 %
Tetratriacontylpentafluropropionate	67.11 %	79.90 %
Tetratriacontyltrifluoroacetate	72.16 %	100 %
Tetradecane, 1 bromo	30.45 %	100 %

50 ^{0}C and 60 ^{0}C). This strain can grow at different pH (6, 8 and 10). The strain was able to grow at 2 %, 4 %, 6 % and 8 % but not at 10 % conc. of NaCl. GD_2 strain showed positive results for (citrate utilization, arginine decarboxylation, nitrate reduction, glucose utilization, xylose utilization and arabinose utilization) whereas negative for (indole production, voges - proskauer, lysine utilization, ornithine decarboxylation, malonate utilization, urease, phenylalanine deamination, H_2S production, ONPG, manitol, inositol, sorbitol, rhamnose, sucrose, lactose, adonitol, raffinose and and salicin utilization) tests. The *Acinetobacter* sp. was found to be capable of utilizing n-alkanes of chain length C_{10}–C_{40} as a sole source of carbon and found to be very effective in degrading hydrocarbons present in crude oil (Holst *et al.,* 2007). GD_2 strain was found sensitive against most of the antibiotics. The strain was resistant towards cefadroxil and sensitive towards (norfloxacin, gentamicin, chloramphenicol, cefuroxime, ciprofloxacin, cefoperazone, ceftazidime, roxithromycin, clarithromycin, co-trimoxazole, netillin, cefaclor, cefotaxime, azithromycin, ampicillin, penicillin, amikacin, sparfloxacin, and sulbactam).

2T engine oil degradation by the GD_2 bacterial strain

To elucidate that the strain GD_2 degrade the hydrocarbons present in 2T engine oil, GC-MS was performed. The gas chromatogram of 2T engine oil extracted from M9 medium inoculated with strain GD_2 at 0 day shows the peaks of hydrocarbons as shown in the Fig 1. The corresponding peak of hydrocarbons decreased after 5 and 10 days. The decrease in hydrocarbons peaks shows the biodegradation of hydrocarbons of 2T engine oil. The degradation of following hydrocarbons present in 2T engine oil (Trans, trans-1-6-dimethylspiro [4, 5] decane, Cis trans-1-6-dimethyl spiro[4, 5] decane, Pyrrolo [1,2-a] pyrazine-1, 4-dione, hexahydro-3-(2-methylpropyl), Octateiacontylpenta fluoropropionate, Octacosyltrifluoroacetate, Triacontylacetate, Tetratriacontylpenta fluropropionate, Tetratriacontyltrifluoroacetate

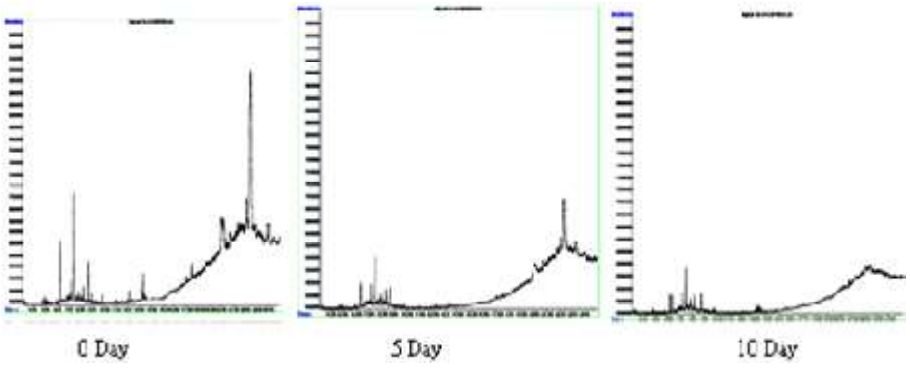

Fig. 1. Gas chromatogram of 2T engine oil extracted from M9 medium inoculated with GD_2 strain at 0, 5 and 10 days

and Tetradecane, 1 bromo) by GD_2 strain after 5, and 10 days was calculated as (24.14, 100 %), (28.57, 100 %), (27.78, 100 %), (43.81, 59.23 %), (37.70, 64.10 %), (48.20, 100 %), (67.11, 79.90 %), (72.16, 100 %) and (30.45, 100 %) respectively as shown in the Table 1. In the present study, from GC-MS analysis it was found that the bacterial GD_2 strain has the ability to degrade 49.91 % and 83.87 % of n-alkanes during 5 d and 10 days of incubation at 37 ^{0}C.

CONCLUSION

The isolated bacterial strain GD_2 was capable to utilize the 2T engine oil as a sole carbon and energy source. Capability to degrade petroleum hydrocarbons makes it potential organism for consideration in the bioremediation of 2T engine oil contaminated sites. However, individual organisms often favor to metabolize a limited range of hydrocarbon substrates. Consequently, a mixed population of bacteria and fungi is usually required to provide all metabolic capabilities for entire degradation of complex mixture of hydrocarbons. Microorganisms able to degrade pollutants ineffective when inoculated into natural environment, so additional research is necessary in this field.

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