Degradation of Polyaromatic Hydrocarbons by Isolated Cultures From Contaminated Soils at Petrol Pump Stations.

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Research Article

Abstract: A laboratory study undertaken to access optimal conditions for biodegradation of hydrocarbon.Among 21 hydrocarbons degredating bacterial cultures isolated from contaminated soil sample collected from petrol pump stations of different places of Latur, Udgir and Solapur. *Bacillus sp,,Acinetobacter spp,Clostridium spp, and Pseudomonas spp.* Were selected for the study based on the efficiency of hydrocarbon i.e.naphthalene/ anthracene in methanol utilization.Phenotypic examination of the recovered bacteria revealed that they belong mainly to the genus *Pseudomonas Spp.Bacillus spp* .Biochemical test were used as an indication for the ability of these bacteria to grow on petrol. Present study indicates that naphthalene and anthracene were degraded.

Key words: Petroleum hydrocarbons,Biodegradattion, hydrocarbon contamination, polluted soils, Microorganisms

Introduction: Industralisation & accidental spillage have increased the pollution of Hydrocarbon compounds in the soil as well as water .The major constituents of contaminated soil are alkanes,cycloalkanes,benzene,substituted benzene and naphthalene. Even though alkanes are most abundant compounds in oil ,it is necessary for microorganisms that are also able to oxidize alkylated polyaromatic hydrocarbons(PAHs).The most widely distributed environmental pollution can be attributed to hydrocarbon contamination.The environmental pollution by hydrocarbons at old petrol stations or factory sites is serious problem as well as not only does the pollution cause damage to the environment but also the sales value of land decreases significantly .physical technologies such as cumbustion & solidification have been carried out to remove hydrocarbons from contaminated soil.Althogh physical techniques may shorten the work period with low costs plants are not able to grow in these soils and it is well known that microbial degradation of spilled hydrocarbons is a major technique in the natural decontamination process therefore ,various bacteria degrading hydrocarbons have been isolated .

Also the wide variety of polycyclic aromatic hydrocarbons are found in the environment as a result of the incomplete cumbustion of organic matter,emission sources,automobile exhausts, stationary matter,domestic matter ,area source matter and also in food.Some PAHs have also been used in synthesis of different organic compounds in pesticides,fungicides,detergents,dyes and mothballs.

Many PAHs have toxic, mutagenic and carcinogenic properties. PAHs are highly lipid soluble and thus readily absorbed from the gastrointestinal tract of mammals. They are rapidly distributed in a wide variety of tissues with marked tendency for localization in body fat

therefore many PAHs are considered to be environmental

pollutants that can have a detrimental effect on the flora and fauna of affected habitats resulting in the uptake and accumulation of toxic chemicals in food chains &in some instances in serious health problems or genetic defects in humans.(4) Naphthalene the first member of the PAH group is a common micropollutant in potable water. The toxicity of naphthalene has been well documented and cataractogenic activity has been reported in laboratory animals.Naphthalene binds covalently to molecules in liver,kidney and lung tissues, thereby enhancing its toxicity, it is also an inhibitor of mitochondrial respiration.

Naphthalene poisoning in humans can lead to hemolytic anemia and nephrotoxicity. In addition dermal and opthalmological changes have been observed in workers occupationally exposed to petrol pump area The first step in microbiological degradation of PAH is the action of dioxygenase which incorporates atoms of O2 at two carbon atoms of benzene ring of PAH resulting in the formation of cis-dihydrodiol which undergoes rearomatization by dehydrogenase to form dihydroxylated intermediates.

In order to protect Environment from such PAH emission from diesel oil ,a stringent EURO111 standard has been enforced.this specifies that the minimum allowable concentration of PAH in diesel oil to be used as automobile fuel should be 11% by weight.conventional hydro treatment of diesel (using co- mo/ni-mo catalysts) to reduce the PAH content below this permissible limit failed.Even under high pressure (80 kPa) and temperature (633 k) conversion of aromatics to naphthenes has so far been achieved only in order of 40%.investigation on the degradation on PAH is being carried out for a long time and despite of the fact ,as observed by some of the investigators,that these compounds may resist degradation by microbial enzymes (7), many papers are appearing in literatures describing the success of biodegradation process of PAH .This relatively new technology demands proper coordination between classical microbiological work and bioprocess engineering ,followed by bioseperation for its successful use in industry.

The objective of study is to assess the hydrocarbon biodegradation potential of selected few bacterial strains which identified by Indrayani Laboratories , Latur and programmed bioprocess study of a mixed culture system capable of degrading PAH from a simulated mixture has been reported. In order to initiate the bioprocess study(3-7) ,the mixed culture from native sources viz.soil of petrol pump of three cities Latur , Udgir and Solapur has been carried out.

Materials and Methods:

Source of Soils

Soils were collected from petrol stations of Latur,Udgir and Solapur in India used for isolation of hydrocarbon utilizing microorganisms.The soil sample were collected in pre-sterilized sample bottles and the samples duly labeled were stored at -4 0 C for further analysis.

Microbiological Methods

Enrichment , Isolation and Identification of Hydrocarbon Degrading bacteria:

A soil sample (1 g) from each site was suspended separately in 20 ml of sterile selective medium (5) in a 50 ml Erlenmeyer flasks containing : KH2PO4(1.000g), Na2HPO4 (1.250g), (NH4)2SO4 (1.000g), MgSO4 .7H2O

(0.500g), Cacl2.6H2O (0.050g) and FeSO4 .7H2O (0.005g). Each soil-medium suspension was supplemented with 100µl of 1% (w/w) solution of naphthalene/anthracene in methanol, sterilized through a millipore membrane filter under positive pressure as normal autoclaving process of such hydrocarbon solution could not be carried out. The flasks were then kept in an shaking incubator at (37oC, 100 rpm) for two days so that the bacteria could adapt to the new laboratory environment. Growth of microorganisms was indicated by visible turbidity of the solution, which was verified by sub-culturing it on to same nutritive bacterial culture medium along with polyaromatic hydrocarbon compounds dissolved in methanol.

Enriched culture was obtained by repeated inoculation of bacterial culture from different places the soils of three cities mentioned earlier, was mixed since it contained different strains of bacteria capable of degrading naphthalene/anthracene into separate flask with fresh selective medium mentioned earlier.After subculturing steps broth was centrifuged at 8,000 rpm for 5 min. and the cell pellets were obtained. These cell pellets were washed with 0.1*M* phosphate buffer solution twice and then

inoculated into selective nutritive bacterial culture medium.Pure hydrocarbon degrading strains were isolated on agar plates which were labeled as LA,UA,SA,LN,UN & SN resp. and incubated at 37oC for one week in an incubator.Pure and representative colonies were transferred to slants for preservation and the isolated bacterial cultures were characterised by their morphological and Biochemical characteristics(2)

Identification of Bacterial culture by Morphological and Biochemical Tests:

The bacterial cultures were classified mainly to their generic level. Morphological identification of the isolated strain was done by gram staining and by motility test. Biochemical tests like oxidase reaction,nitrate reduction, decarboxylases, catalase test,oxidative-fermentative test, citrate, gelatin liquefaction test, indole test, TSI (Triple Sugar Iron Agar Test), Methyl Red test, malonate test, phenylalanine deaminase test, Voges Proskauer test,Dnase test, Mackonkey test, xylose-, sucrose-, manitol-, mannose tests, growth in blood agar, growth at 42oC, esculin hydrolysis, lysine, urease, antibioticsensitivity, antibiotic resistance, were also performed (2-6).

The details of all the tests and names of bacteria are given in Table 1.

It was observed that the isolated strain *Pseudomonas alcaligenes* from Latur petrol pump area soil degrades naphthalene most efficiently among the strains of all cities, whereas *Serratia rubidaea(SA2)* isolated from solapur region degrades anthracene, respectively. Thus, pure cultures of these bacteria were used for further investigations.

Photograph 1: Hydrocarbon degrading isolated

Photograph 2: The Strip used during automation for Biochemical Tests for identification of microorganisms

Experimental Methods

The broth of selective nutritive medium prepared and inoculated with preincubated pure culture suspension which placed on shaking incubator for two days at 37oC, 100 rpm. and O.D. were taken after every two days interval observed change in O.D. has shown degradation of hydrocarbons used. Then content of each flask was centrifuged and the bacterial mass and the aromatic content were determined.

Table 1 : Microbiological Tests of Microorganisms Isolated from Soil of Latur and Solapur Cities:
[Abbreviation : + = positive, – = negative] (24)

	Pseudomonas alcaligenes(LN3)	*Serratia rubideae* (SA2)
1. Media used for inoculation	Selective nutritive media+ naphthalene	Selective nutritive media + anthracene
2. Colony character on nutrient agar	Yellowish,Smooth,Opaque	Red,Smooth,Opaque
3. Gram's nature	Gram-negative rods	Gram-negative bacillococci
4. Motility	Motile	Motile
5. BIOCHEMICAL TESTS:		
a) Oxidase reaction	-	+
b) Sugar Test	-	+
c) TSI Test	+	+
d) Lysine Test	-	+
e) Nitrate reduction test	+	+
f) Citrate Test	+	-
g) Arginine Test	+	-
h) Gelatin liquification Test	-	+
i) Urease Test	-	+
j) Catalase Test	+	+

Results and Discussion:

As mentioned earlier, the present study involves biodegradation of polyaromatic hydrocarbons by using spectrophotometric method and coloum chromatography.Initially microorganisms utilizes the specific substrates naphthalene/ anthracene for its own growth and later when an appreciable quantity of biomass is formed, a biodegradation takes place through specific biocatalysis a biodegradation takes place in order to study reaction by performing morphological & biochemical tests and compare with bergey's manual we obtained two efficient strains it is evident that the reaction engineering behaviour of the degradation of hydrocarbon studied.

The bacterial cultures were classified mainly to their generic level ,morphological identification of the isolated strain were done by biochemical tests like nitrate reduction ,catalase test ,oxidative fermentative ,citrate,gelatin liquification,TSI,sucrose,lysine ,urease tests were also performed.The details of all these tests and names of bacteria are given in table.1 and it was observed that the isolated strains,collected from Solapur ,degrades hydrocarbons most efficiently among the isolated strains of Latur city.Among the isolated strains *pseudomonas alcaligens* degrades naphthalene whereas *serratia rubidaea* species degreades anthracene.*Serratia rubideae* is bacillococci degrades polyaromatic hydrocarbons efficiently but also found to be a rare invasive pathogen causing urinary tract infection. Thus,pure cultures of these bacteria were used for further investigations.

Conclusion:

The increasing incidents of oil spills as well as pollution of soil around filling stations demands the degradation of complex hydrocarbons. The complex hydrocarbons which are otherwise harmful to aquatic flora fauna, harmful to ecosystem must be degraded to simpler hydrocarbons which are not harmful. This method of degradation involves use of bacteria which grow on polyaromatic hydrocarbons and utilizes it. so ,the method is biological and safer to ecosystem than other methods.

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