

Isolation and Characterization of Hydrocarbon Degrading Bacteria's isolated from Diesel Polluted Soil from Various Petrol-Diesel Bunk of Solapur

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Research Article

Abstract: Research work deals with two aspects, firstly collection of diesel polluted soil samples from various petrol-diesel bunks from Solapur, secondly it deals with the laboratory work which includes culturing and sub-culturing to get pure culture of micro-organisms from soil sample, and study regarding characterization and degradation of hydrocarbons. Gram staining, sugar fermentation, biochemical and IMVIC test were carried out on isolated micro-organism which revealed the isolated micro-organisms were from *Bravibacterium* spp., *Streptococcus* spp., *Bacillus* spp., *Enterobacter* spp. Research work indicates that these isolates were capable to degrade hydrocarbons like diesel, anthracene, naphthalene and benzene.

Keywords: Isolation, hydrocarbon degradation, Diesel, Anthracene, Naphthalene, Benzene.

Introduction

Day by day the use of petrol and diesel is increasing, with the use, its transportation, disposal is also increasing which is leading to increase in its spillage during transportation and over soil during disposal causing soil pollution ultimately leading to environment pollution. Last and current century is an machine age and using petrol and diesel as energy source for automobiles which are giving rise to spillage during their disposal in machines. Diesel fuel is principle end product of gas obtained during fractional distillation of petroleum as the portion boiling off between 25°C and 36°C (Atlas 1995). Diesel oil is a medium distillate of petroleum containing: n-alkanes, branched alkanes, olefins and small concentration of aromatic polycyclic compounds (Baker and Herson, 1999). Bioremediation processes have been found to be an efficient method for remediation of petroleum by-products, pesticides and other potential harmful chemical (Castro-Gutierrez et al., 2012). Bioremediation is being used or proposed as a treatment option at many hydrocarbon contaminated sites (Braddock et al., 1997). Bioremediation processes are significantly affected by the inherent capabilities of the

microorganisms, their ability to overcome the bioavailability limitations in multiphase environment scenarios(oil-water-soil) and environmental factors such as temperature, pH, nutrients and electron acceptor availability (Mukherji and Vijay, 2002). Environmental microorganisms with the ability to degrade crude oil are ubiquitously distributed in soil and marine environments (Venkateshwaran and Harayama, 1995). Diesel oil spills on agriculture land generally reduce plant growth and reasons for the reduced plant growth in diesel oil contaminated soils range from direct toxic effect on plants and reduced germination (Udo and Fayemi, 1975). Microorganisms have enzyme systems to degrade and utilize diesel oil as a source of carbon and energy (Ijah and Antai, 1988; Ezeij et al., 2005; Antai and Mgbomo, 1993). Biostimulation is consider as a most appropriate remediation technique for diesel removal in soil and requires the evaluation of both intrinsic degradation capacities of the autochthonous microflora and the environmental parameters involved in the kinetics of the in situ process. Polyaromatic hydrocarbons can cause mutagenesis and cancer. They are readily absorbed by gastrointestinal tract of mammals as they are highly lipid soluble. Thus due to their toxic nature they are considered to be environmental pollutant and have a detrimental effect on the flora and fauna of affaceted habitat resulting in the uptake and accumulation of toxic chemicals in food chains and in some instances in serious health problems or genetic defects in humans.

Materials and Methods

Materials

Instruments

1. Incubator
2. Rotary shaker
3. Digital weighing balance

4. Autoclave

5. PH meter

6. Microscope

Glass Wares

1. Conical flasks

2. Pipettes

3. Petri plates

4. Test tubes

5. Saline tubes

6. Beakers

7. Glass slides

8. Cavity slides

9. Glass spreader

Other Requirements

1. Surface sterilizer

2. Cotton

3. Bunsen burner

4. Nichrome wire loop

Method

1. Collection and screening of sample

The soil samples (100g each) were collected from various diesel spilled stations for the isolation of oil degrading microorganisms. The samples were collected in pre-sterilised glass bottles and transported to the laboratory for analysis.

Enumeration and isolation of hydrocarbon degrading bacteria was carried out through serial dilution-agar plating technique using basal salt mineral agar media (BSM) - KH₂PO₄: 0.38g ; MgSO₄·7H₂O: 0.20 ; NH₄Cl: 1g ; NaH₂PO₄: 1g ; Peptone: 1g ; Distilled Water: 1000ml.(A. M. Deshmukh, 1997)

2. Isolation and screening of hydrocarbon degrading bacteria

Preparation of four set of basal salt mineral agar media was prepared which was respectively inoculated by 0.1% of hydrocarbons viz. Diesel, Anthracene, Naphthalene and Benzene. 1g of soil sample was weighed and added in 10ml of distilled water and serial dilution was carried out till 10⁻⁵. From 10⁻⁵ dilution tube, 0.1ml of dilution was pipetted out in 0.1% hydrocarbon containing BSM agar

2. Morphological studies cum Gram staining of isolates

	Shape	Colour	Margin	Opacity	Elevation	Consistency	Gram's nature	Motility
A	Circular	Orange	Regular	Opaque	Convex	Moist	Capsulated Gram positive bacilli	Motile
B	Irregular	Yellowish	Irregular	Opaque	Convex	Moist	Gram positive cocci in chains	Non-motile
C	Circular	White	Regular	Opaque	Convex	Moist	Gram positive rod shaped spore former	Motile
D	Irregular	White	Irregular	Opaque	Convex	Moist	Short pink rod Gram negative	Motile

media. Isolation of colonies were carried out by pour plate method. Plates were incubated for 7 days. The well developed colonies were selected and pure culture was obtained on Basal salt mineral agar medium. The further characterization of obtained pure culture was carried out by Gram's staining, biochemical test, sugar fermentation test and IMVIC test.

3. Characterization of bacteria.

The isolates were grouped to various genera as per Bergey's Manual of Determinative bacteriology. These cultures were characterised depending on their morphology, gram staining, spore staining, mobility, oxidase, catalase, oxidation fermentation, gas production, ammonia formation, nitrate and nitrite reduction, indole production test, methyl-red and Voges-Proskauer test, citrate and mannitol utilization test, hydrolysis of casein, gelatin, starch, urea and lipid.

Observation and Result**Observation**

1. Images of four different pure culture that were grown Basal salt mineral agar media containing 0.1% Diesel.



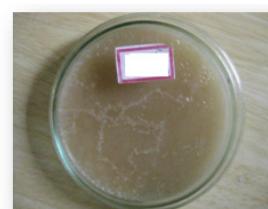
Pure culture A



Pure Culture B



Pure Culture C



Pure Culture D

3. Biochemical test

Test	A	B	C	D
Urease test	+	+	+	+
Glucose	--	+-	++	+-
Maltose	--	+-	++	+-
Lactose	--	+-	+-	++
Sucrose	++	+-	++	+-
Nitrate Reduction Test	+	+	+	+
Starch Hydrolysis	-	-	-	-
Phenyl Alanine Deaminase Test	-	-	-	-
H2S Production	-	-	-	-
Mannitol	--	+-	+-	--
Catalase	+	+	+	+

(++) = Acid Production and Gas Production

(+) = Acid Production

4. IMVIC test

Isolates	Indole	MR	VP	Citrate
A	-	+	+	-
B	+	+	+	-
C	+	+	+	-
D	-	+	+	+

(+) = Positive test

(-) = Negative test

Result

From the soil with oil spills we got four isolates having ability to degrade the hydrocarbons on Basal Salt Mineral agar medium. The colony characteristics and Gram nature of these isolates were studied also the biochemical tests were performed. First (A) isolate shows the synthesis of orange color pigment and motile, Gram positive nature. Second (B) isolate shows the yellowish colony color, non-motile in nature, gram positive nature, the cocci were arranged in short chains. The third (C) isolate shows white color colony, the cells are motile in nature, which are Gram negative in nature and also are spore former. The fourth (D) isolate shows white color colony, cells are motile with Gram negative in nature. Then by using Bergey's manual of bacteriology volume I & II, the species identification of isolates was carried out according to the morphological characteristics, Gram nature, results of biochemical and IMVIC test.

As a result four different species of hydrocarbon degrading bacteria were identified as,

1. *Bravibacterium* species
2. *Streptococcus* species
3. *Bacillus* species
4. *Enterobacter* species

CONCLUSION

Day to day increase in use of hydrocarbons such as petrol, diesel, is also contributing to increase in pollution. This pollution is harmful to nature as well as animals. It's a necessary to degrade these hydrocarbons. The bacteria which are growing in soil which are polluted by such hydrocarbons have ability to degrade these hydrocarbons,

thus these bacteria's can be used to degrade hydrocarbons.

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