iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 4 **Issue:** V **Month of publication:** May 2016

DOI:

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)

Biodegradation of Petrochemicals by Microorganisms Isolated From Garage Soil of Gwalior Area

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Abstract--Bioremediation functions basically on biodegradation, which may refer to complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds, and cell protein or transformation of complex organic contaminants to other simpler organic compounds by biological agents like microorganisms. Many microorganisms are present in water and soil are capable of degrading hydrocarbon contaminants. This paper presents an overview of petrochemicals degradation by microorganisms. This work was carried out to determine the microbial flora of soils contaminated with used petrochemicals in Gwalior. Soil samples were collected from different mechanic workshops of Gwalior city. Isolated bacteria belong to genus Bacillus and Pseudomonas from soil samples showed optimum growth in presence of hydrocarbons like petrol. These isolates can be applied for the bioremediation of petrochemical hydrocarbons including waste water, which would ultimately lead in making eco-friendly.
Key words: Bacterial species, Garage soils, Petrochemicals

I. INTRODUCTION

Today era is facing one of the major problems regarding oil contamination. It can create a threat to our environment. Now it's the time for an environmentalist to deal with this problem very seriously. Petrol engine oil, which is one of the major products of crude oil, constitutes a major source of pollution in our environment. The main consumption of petrol engine oil is vehicles and generators. Major stored part of petroleum hydrocarbons can be seen in garage where daily lot of petroleum deposited. Petroleum-based products are the major source of energy for industry and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products. The amount of natural crude oil seepage was estimated to be 600,000 metric tons per year with a range of uncertainty of 200,000 metric tons per year [1]. Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution [2]. Soil contamination with hydrocarbons causes extensive damage of local system since accumulation of pollutants in animals and plant tissue may cause death or mutations [3]. The technology commonly used for the soil remediation includes mechanical, burying, evaporation, dispersion, and washing. However, these technologies are expensive and can lead to incomplete decomposition of contaminants.

The process of bioremediation, defined as the use of microorganisms to detoxify or remove pollutants owing to their diverse metabolic capabilities is an evolving method for the removal and degradation of many environmental pollutants including the products of petroleum industry [4]. In addition, bioremediation technology is believed to be noninvasive and relatively cost-effective [5]. Biodegradation by natural populations of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment [6] and is cheaper than other remediation technologies [7].

This study was therefore designed to monitor the rate of biodegradation of petrol engine oil (hydrocarbon) by microorganisms isolated from garage soil (petroleum contaminated soil).

II. MATERIAL AND METHODS

A. Preparation Of Modified Petrol Medium

The modified petrochemical medium comprised of 0.7 gm K2HPO4, 0.1 gm (NH4)2SO4, 0.3 gm KH2PO4, 0.3 gm MgSO4 7H2O, 2.2 gm agar – agar. The mineral components of the medium were dissolved in 100 ml of distilled water and mixed with 2 ml of petrol engine oil . The medium was autoclaved at 121^{o}C for 15 min.

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B. Enrichment Of Microorganisms

Soil sample collection and preparation: Top surface soil sample was collected from garage of Gwalior area (Shinde ki chhavani (S1) and Phoolbagh (S2)) in sterilized plastic containers. Soil sample meant for degradation studies was sterilized using autoclave at 121^{o}C for 15 min, after which it was allowed to cool to room temperature for further treatments. Microorganisms capable of degrading petrol engine oil were enriched in sterile modified petrol engine oil medium by inoculating soil (which was collected from garage) in to the medium in 250 ml conical flask. 0.5 gm of this garage soil was inoculated in to the 100 ml of sterile modified petrol oil broth and allowed to incubate at 37^{o}C for 1 week.

C. Isolation Of Microorganisms

After 1 week of incubation period, 1 drop of enriched culture was spread on to the sterile modified petrol oil agar plate. The plate was incubated at 37^{o}C for 48 hr. After 48 hr incubation; two different bacterial colonies were selected from incubated plate. Each bacterial colony type was sub cultured repeatedly onto sterile nutrient agar plates to obtain a pure culture. Pure cultures of bacterial isolates were identified on the basis of their colonial morphology and biochemical characteristics.

D. Determination Of Microbial Colony Numbers For Degradation Studies

5 ml of sterile Nutrient broth was aseptically inoculated with a loopful of pure culture of Colony 1(P1) in first test tube and Colony 2 (P2) in second test tube and incubated both the tubes at 37^{o}C for 24 hr. After incubation, the numbers of organisms present in one ml of nutrient broth were determined by spread plate method. The numbers of organisms were adjusted in both the tubes in such a way that both the isolates contain approximately equal numbers of microorganism in one ml of sample by using sterile Nutrient broth as a diluent7. Soil sample collection and preparation: Top surface soil sample was collected from garage of Gwalior area (Shinde ki chhavani (S1) and Phoolbagh (S2)) in sterilized plastic containers. Soil sample meant for degradation studies was sterilized using autoclave at 121^{o}C for 15 min, after which it was allowed to cool to room temperature for further treatments.

Description and treatment of samples: Test:

S1 samples of 15 gm sterilized soil mixed with 1 ml (0.848 gm) of Sterile petrol engine oil + 0.2 ml culture of P1,

S1 samples of 15 gm sterilized soil mixed with 1 ml of Sterile petrol engine oil + 0.2 ml culture of P2,

S2 samples of 15 gm sterilized soil mixed with 1 ml of Sterile petrol engine oil + 0.2 ml culture of P1,

S2 samples of 15 gm sterilized soil mixed with 1 ml of Sterile petrol engine oil + 0.2 ml culture of P2,

Control: 5 samples of 15 gm sterilized soil mixed with 1 ml (0.848 gm) of Sterile petrol engine oil + 0.2 ml of sterile distilled water.

Petrol oil degradation studies: The ability of P1, P2 and mixture of both the bacterial isolates to degrade petrol oil was monitored on the first day (day zero) of the study and subsequently at 5-day interval for 25 days. Carbon tetrachloride was employed as an extractant. On each day, two samples per single treatment were analyzed for the quantity of residual petrol oil. Each of the 15gm soil treatment samples was mixed with 40 ml of carbon tetrachloride, placed in a separating conical flask, shaken vigorously for 3 min and allowed to settle for 5 min. The liquid phase was separated by allowing the supernatant (petrol oil – carbon tetrachloride) to pass gradually through a funnel fitted with filter paper (Whatman No 1). Anhydrous sodium sulphate spread on the filter paper was employed to remove any moisture in the mixture. The liquid phase was collected in a 50-ml pre-weighed beaker. The beaker containing the extract was placed in an oven and the extractant allowed to evaporate at 50oC. The beaker with the residual petrol oil was allowed to cool to room temperature and weighed to determine the quantity of residual petrol oil by difference8.

III. RESULTS AND DISCUSSION

The soil samples were gathered from the garage (petrol contaminated site) to isolate the native bacterial population that have potential to utilize crude oil hydrocarbons present in contaminated sites, was confirmed by this present study. Hydrocarbonoclastic organisms isolated from garage soil were studied for further identification . Table – 1 & 2 and figure 1 & 2 shows that, using cultural characteristics and biochemical characteristics, two bacterial isolates were found to be *Bacillus* sp. and *Pseudomonas* sp. (As were identified by comparing it with the Bergey's manual of determinative bacteriology). Isolates of *Bacillus* sp and *Pseudomonas* sp., and Mixture of both the culture showed different abilities in the breakdown and utilization of the petrol engine oil.

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Volume 4 Issue V, May 2016
ISSN: 2321-9653
International Journal for Research in Applied Science & Engineering Technology (IJRASET)

Table-1 Colony characteristics of bacterial isolates on Nutrient agar plate :

S.No	Size	Shape	Elevation	Color	Consistency	Opacity
P1	1.5-2.2mm	Circular	Conx	Creamiest white	Butyrous	Opaque
P2	3.2 – 3.5 mm	Irregular	Flat	Fluorescent green	Mucoidal	Translucent

Table 2: Identification of petrochemical hydrocarbon (Petrol engine oil) degrading bacterial isolates

Characteristics	*Bacillus*	*Pseudomonas*
Colony Characteristics		
Shape	Round	Round
Size	Small	Large
Colour	White	Green
Surface	Dull, granular	Convex
Margin	Entire	Undulate
Morphology		
Straight rod	+	+
Cocci	-	-
Gram stain	+	-
Cell arrangement	Short rod, Single	Short chain, Single
Spore	C	C
Motility	+	+
Enzyme production		
Amylase	+	-
Lipase	+	+
Gelatinase	+	-
Carbohydrate Fermentation		
Glucose	A/-	-
Fructose	A/-	-/-
Sucrose	A/-	-/-
Lactose	A/-	-/-
Mannitol	A/-	-/-
Urease	-	-

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Volume 4 Issue V, May 2016
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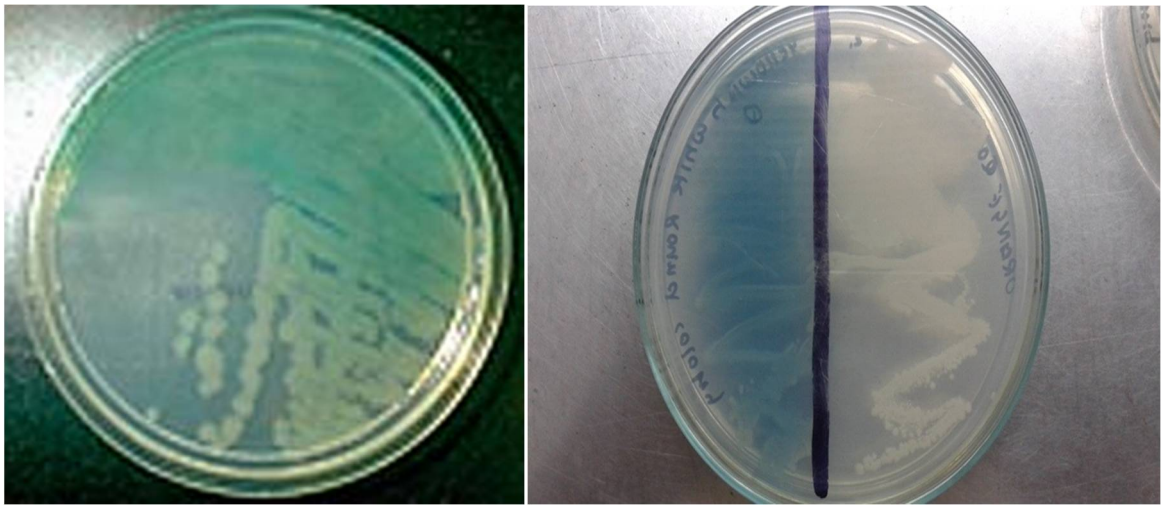
Casein	+	-
Nitrate reduction	+	+
Citrate	+	+
Oxidase	+	+
Catalase	-	+
Indole production	-	-
Methyl Red	-	-
Voges Proskauer	+	-
Citrate Utilization	-	+

Figure 1 and 2 confirms the bacterial isolates as *Bacillus* sp. and *Pseudomonas* sp.



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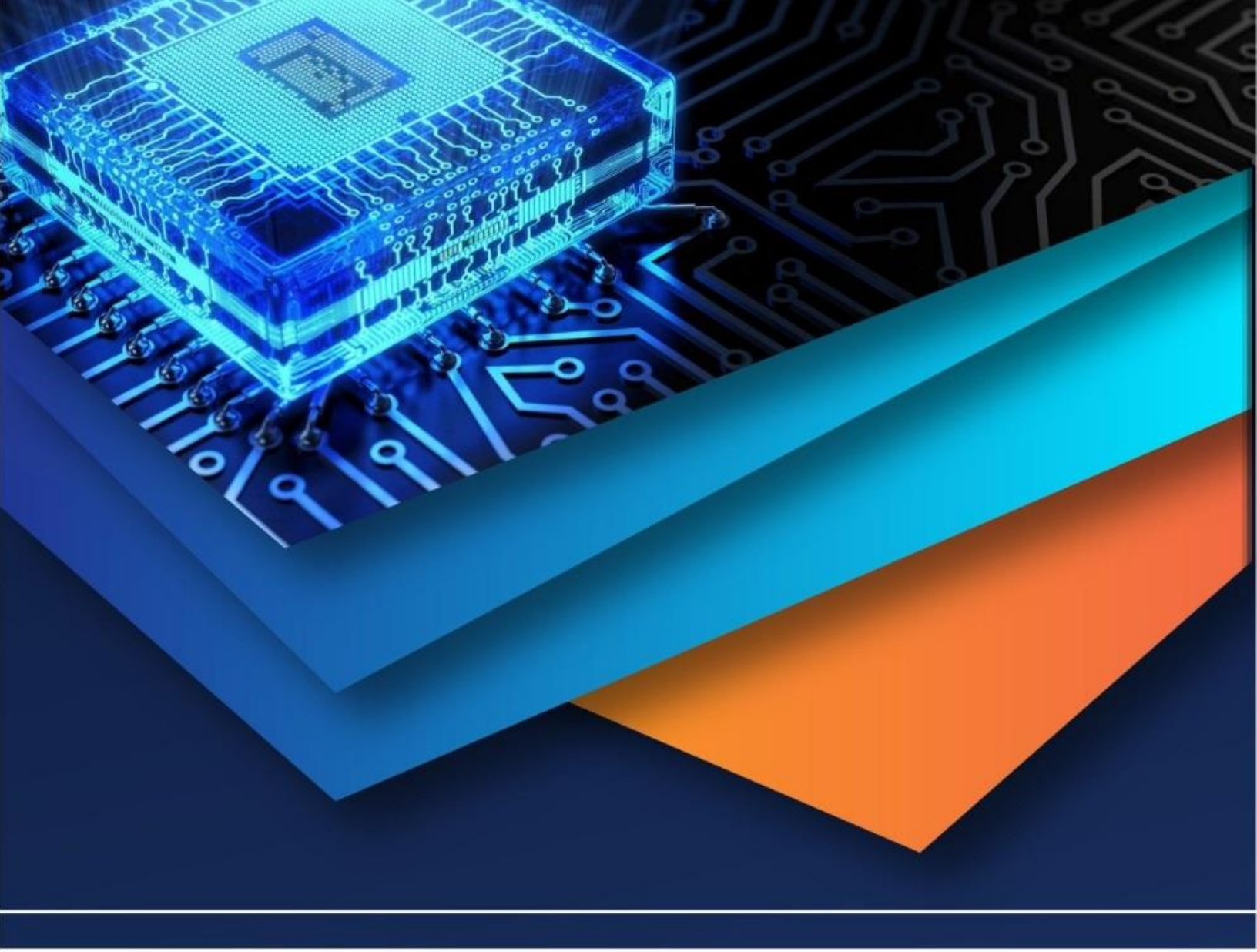

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