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Presence of Hydrocarbon Degrading Bacteria in Contaminated Soil Collected From Various Fuel Station in Bhilai, Chhattisgarh

Lumeshwari[1], Laleeta[2], G. K. Chandrol[3], Priyanka Sahu[4], Manmita Singh Rajput[5]
[1]*Govt. V.Y.T. PG Autonomous College Durg*
[2, 3, 4, 5]*Kalyan P.G. College, Bhilai Nagar*

Abstract: ***In this study, we isolated seven strains (termed BY1–7) from polluted soil at an oil station and evaluated their abilities to degrade total petroleum hydrocarbons (TPHs). Among 45 bacterial colonies one bacterial strain was identified based on the cultural, morphological and biochemical characteristics. The isolated bacterium was then subjected to a preliminary assessment of their crude oil after 48 hours of incubation on nutrient agar plates overlaid with 100 ML of petroleum crude oil, the zone of clearance was observed. The isolated bacteria showed 35% petrol degradation, whereas a relatively high oil degradation rate, almost 40% was observed when the bacterium was acclimatized. The selected bacterial strains crude oil resistance was analysed based on the growth ability on the crude oil containing mediums. This strain was identified as Brevibacterium brevis. After inoculation, growth ability was measured and the highest percentage of petrol degradation occurred at temperature 37 °C with the value 30.8%. Bacteria displaying such capabilities are often exploited for the bio-remediation of petroleum oil contaminated environments. Recently, microbial remediation technology has developed rapidly and achieved major gains. However, this technology is not omnipotent. It is affected by many environmental factors that hinder its practical application, limiting the large-scale application of the technology.***

Keywords: Petroleum hydrocarbon-degrading Bacteria, Petroleum oil, Bio-remediation, Bacterial consortia, Environmental factors, Enzymes.

I. INTRODUCTION

Petroleum oil is an important strategic resource, for which all countries compete fiercely (Sun, 2009). Indeed, anthropogenic activity is reliant on oil to meet its energy demands, which causes the petro-chemical industry to flourish. However, petroleum use results in environmental deterioration (Xue et. al., 2015). During petroleum production, storage and transportation, refining and processing, as well as spills and discharges of petroleum hydrocarbons often occur as a result of blowout accidents during oil field development, leakage from oil pipe lines and storage tanks, oil tanker and tanker leakage accidents, oil well waxing, and during overhauls of refineries and petro-chemical production equipment (Chaerun et. al., 2004; Chen et. al., 2015; Wang et. al., 2018). Large spills should be recycled or eliminated to as great a degree as possible, but in some cases it is difficult to recover the spilled materials, resulting in its remaining in the affected area, and posing persistent risks to the environment.

Micro-organisms therefore represent a promising, largely untapped resource for new environmental biotechnologies. Research continues to verify the bio-remediation potential of micro-organisms. Even dead microbial cells can be useful in bio-remediation technologies. These discoveries suggest that further exploration of microbial diversity is likely to lead to the discovery of many more organisms with unique properties useful in bio-remediation. Microbes able to degrade the contaminant increase in numbers when the contaminant is present. The use of micro-organisms is not limited to one field of study of bio-remediation, it has an extensive use. Oil slicks caused by oil tankers and petrol leakage into the marine environment and oil contaminated waste water are now a constantly occurring phenomenon.

II. PETROLEUM HYDROCARBON-DEGRADING BACTERIA

Most petroleum hydrocarbons encountered in the environment are ultimately degraded or metabolized by indigenous bacteria because of their energetic and carbon needs for growth and reproduction, as well as the requirement to relieve physiological stress caused by the presence of petroleum hydrocarbons in the microbial bulk environment (Hazenet et. al., 2010; Kleindienst et. al., 2015a). The development of microbial biotechnology and high-throughput sequencing technology, such as micro-fluidic techniques (Jiang et. al., 2016; Guerra et. al., 2018), is beneficial for screening and identifying functional micro-organisms from petroleum hydrocarbon contaminated environments.

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Indeed, many studies have revealed that there is a large number of hydrocarbon-degrading bacteria in oil-rich environments, such as oil spill areas and oil reservoirs (Hazen et. al., 2010; Yang et. al., 2015), and that their abundance and quantity are closely related to the types of petroleum hydrocarbons and the surrounding environmental factors (Fuentes et. al., 2015; Varjani and Gnansounou, 2017). Many normal and extreme bacterial species have been isolated and utilized as biodegrades for dealing with petroleum hydrocarbons. The degradation pathways of a variety of petroleum hydrocarbons (e.g., Aliphatic and Poly-aromatics) have been shown to employ oxidizing reactions; however, these pathways differ greatly because of the specific oxygenase found in different bacterial species.

For instance, some bacteria can metabolize specific alkenes, while others break down aromatic or resin fractions of hydrocarbons. These phenomenons suggest that these micro-organisms are crucial to the degradation of petroleum hydrocarbons and that they significantly influence the transformation and fate of petroleum hydrocarbons in the environment. Although some bacteria have been reported to have a broad spectrum of petroleum hydrocarbon degradation ability, *Dietzia spp.* DQ12-45-1b utilizes n-alkenes (C6–C40) and other compounds as the sole carbon sources (Wang et. al., 2011) and Achromobacter xylosoxidans DN002 works well on a variety of non-aromatic and poly-aromatic hydrocarbons (Ma et. al., 2015), almost no bacteria can degrade the entire petroleum hydrocarbon fraction. Indeed, most bacteria can only effectively degrade or utilize certain petroleum hydrocarbon components, while others are completely unavailable (Chaerun et. al., 2004; Varjani, 2017).

III. MATERIAL AND METHOD

Micro-organisms were isolated by “Selective Enrichment Technique”. The commercial petroleum (crude) oil was used for enrichment and bio-degradation experiments as the sole carbon and energy source for micro-organisms. Bushnell-Hass (BH) broth medium (Bushnell and Haas, 1941) was used in enrichment technique supplemented with hydrocarbon source. The broth medium containing soil and crude oil was incubated at 22ºC with orbital shaking (120R/min). Enrichment of microbial culture was carried out in 300 ml Erlenmeyer flasks containing 100 ml of media. The pH was adjusted to 7.3. The broth medium was sterilized by autoclaving (121ºC for 20 min).

The original soil sample (01g) was added to 100 ml of broth medium containing 01g of crude oil. 0.1 ml of broth is then taken from the 100 ml media and spread plated on nutrient agar medium with 100 micro-liter petrol. Total 09 distinct colonies were obtained from which one with prominent growth was chosen. The Bushnell-Hass (BH) medium consists, per liter, of: 0.2 g $MgSO_4$, 0.02 g $CaCl_2$, 1.0 g K_2HPO_4, 1.0 g KH_2PO_4, 1.0 g NH_4NO_3, 0.05 g $FeCl_3$, pH 7.0. Bushnell-Hass (BH) medium was supplemented with 1% (v/v) diesel as the sole carbon source.

Aliquots of groundwater (03 ml) were added to 250 ml Erlenmeyer flasks containing 100 ml sterile Bushnell-Hass medium supplemented with diesel and the flasks were incubated at 30 °C for 7 days on rotary shaker (150 rpm). Then, 03 ml aliquots were taken and inoculated in fresh Bushnell-Hass medium and incubated in same conditions. After a series of six further subculture, 01 ml aliquot was taken from the medium, diluted in sterile saline solution (0.85% w/v) and plated on Nutrient Agar (NA) medium (Himedia Labs) for incubation at 30 °C. After 48 hours, phenotypically different colonies were picked out for purification on NA medium. The NA medium consists, per liter, of: 5.0 g peptic digest of animal tissue, 5.0 g NaCl, 1.5 g beef extract, 1.5 g yeast extract, 15.0 g agar, pH 7.4 ± 0.2.

IV. BACTERIAL IDENTIFICATION

The randomly isolated bacterial strains from the samples were identified up to generic level by employing the standard morphological and bio-chemical characteristics described in Bergey’s manual of systematic bacteriology (Sneath et. al., 1986).

V. RESULTS AND DISCUSSION

During isolation and characterization, 45 hydrocarbon-degrading bacterial colonies were isolated from enrichment cultures. All isolates were able to completely decolorize the DCPIP-BH medium with petroleum indicating the degradation ability. Two strains (arbitrarily named as L26 and L30), however, decolorized faster the medium supplemented with petroleum (within 72 hours). These strains also completely decolorized the DCPIP-BH medium after 24 hours when hexadecane was used as hydrocarbon source and the medium was completely decolorized or the color has changed after 21 days when benzene, toluene or xylene was used.

Pseudomonas sp., Mycobacterium cosmeticum, Enterobacter sp., Staphylococcus sp., Lysinibacillus sp., Bacillus sp., Pseudomonas sp., *Brevibacterium brevis*

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Table: - Biological Characteristics of Isolation.

S. NO.	TEST PARAMETERS	OBSERVATION
1.	Morphology	Rod shaped
2.	Grams staining	Gram positive
3.	Gas production	negative
4.	Indole test	Negative
5.	Catalase test	Positive

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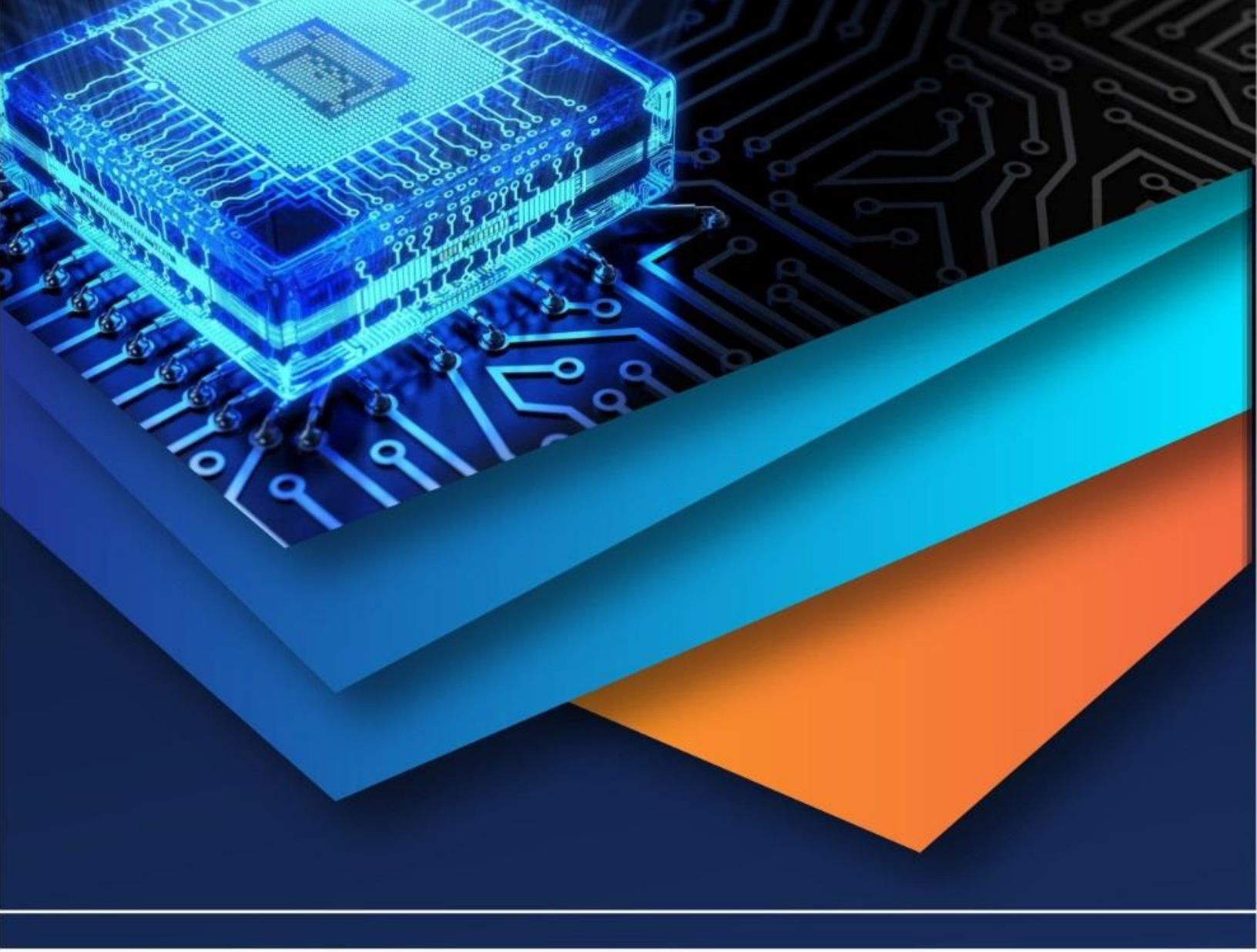
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