ORIGINAL RESEARCH PAPER

Medical Science

# INVESTIGATION OF OIL DEGRADING ABILITY OF BACTERIA ISOLATED FROM SOIL.

**KEY WORDS:** oil degradation, spillage, environment, crude-oil.

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**ABSTRACT**

Human induced calamities have affected the nature in most adverse ways. Oil spills are one such phenomenon that happens due to technical mishaps in the mid-ocean oil rigs or due to accidents. The spilled hydrocarbons cause death of marine lives that come in contact with the oil. Some bacteria have the capability to assimilate these hydrocarbons by feeding on them. The current study aims to isolate oil degrading bacteria from soil and to test the degrading ability of the isolated bacteria on different types of oils.

**Introduction:**

Oil contamination of environmental resources has been a global concern since decades. It has worried most of the environmentalists worldwide. Incidents of oil spills in oceans due to accidents or any other reasons, drastically disturbs the marine life, which includes planktons, fishes, seabirds, marine mammals and reptiles, corals, sea grasses, saltmarshes, mangroves etc. as reported by The International Tanker Owners Pollution Federation Limited in the Technical Information Paper, 2011. The oil generally spreads to vast area that may span across hundreds of squares of miles. It can lead to death of fishes, phytoplanktons, zooplanktons and other aquatic organisms, leading to environmental imbalance. 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. Not only due to accidents, the crude oil seepage from the mid-ocean oil rigs also poses threat to marine life. The seepage is estimated to be 6,00,000 metric tons per year (Kvenvolden K. A. and Cooper C. K., 2003).

Ever since the fuel industry has started in the world, there have been several incidents of oil spills all over the world, due to accidents or leakage from the oil rigs into the water bodies. The spilled oil reduces the light penetration into the water that affects the lives of phytoplanktons. The fishes and other organisms ingest the hydrocarbons which make them seriously ill and ultimately cause their death. This has very serious implications on the aquatic food web in that area of spill if the oil is not removed quickly. Not only in water bodies, the oil spill problem also persisted in the terrestrial environment. The oil if spilled cuts the air supply to the micro-biome of the affected area, leading to loss or damage to vegetation. However, some microorganisms survive the excess of oil by feeding on the oil itself. These microbes can be used to handle the problem of oil spill naturally and rapidly.

The incidents of oil spill are not limited to oceans but, have also been reported in fresh water systems (**Steen** *et al.*, 1999). This can lead to epidemic if the affected water body is used for water supply in the nearby cities. Non-spill-related petroleum by-products (tarballs) that are discharged into rivers also posses threat to environment. Sea turtles are the most adversely affected by this. Oil exposure is therefore a threat to sea turtles both in the presence and in the absence of a reported spill. Oil spill in terrestrial area can also harm agriculture in that area. A study conducted in Nigeria proved that the hydrocarbons generally imply low soil fertility, which in turns implies low agricultural productivity and reduce source of livelihood in the affected areas. Salinity of the affected area increase significantly (Isama L R and Isama L, 2013). Studies suggest that hydrocarbons accumulate in the blubber, liver, kidney, and other organs of seals, sea lions and walruses due to long exposure of oil. It also causes eye irritation and damage (Jacqueline M and Merv F, 2015).

In Bharat, recently, oil has spilt in the coast line of Chennai port due to accident between an oil tanker and an LPG tanker off Kamarajar Port in Ennore, in Chennai on January 30, 2017. The INCOIS (Indian National Centre for Ocean Information Services) reported that the spillage has polluted 24.06 km of the Chennai's coastline. It has affected the fishermen drastically. Subsequent reports by the Coast Guard have since estimated that over 20 tonne of oil have spilled into the Bay of Bengal. An article recently published in The Hindu reported that oil degrading strains have been isolated by scientists at Kunnur University. The three new strains including two species of *Burkholderia* and one species of *Pseudomonas* have been sequenced and submitted to the Genbank database on organisms.

Bioremediation is the most effective process to eliminate the crude oil contaminations from the area of spill. In this process, the affected area is dumped with microorganisms that feed on hydrocarbons, thus clearing up the oil from the site of spill. Microbes, particularly bacteria such as *Pseudomonas sp.*, *Arthrobactera sp.* etc. consume or break the complex hydrocarbons, of which oil is generally made of, into simpler molecules that can be assimilated by the marine lives. Many bacteria have been isolated, that have the ability to degrade hydrocarbons in the marine oil spills (Lies I S, 2007). Bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia, Bacillus cereus* etc. isolated from various sources have been reported to successfully degrade hydrocarbon, particularly diesel (AlDisi *et al.*, 2016). Arpita G and Sheetal S (2015), in a study found that the *bacillus cereus* expressed better oil emulsification and degradation ability. This could be suggested that the *bacillus cereus* strain can be effectively used in the bioremediation of oil pollution. The genome analysis of *Pseudomonas aeruginosa,* isolated from the oil spill area of Assam, revealed versatility for degradation, emulsification and metabolizing of crude oil (Das *et al.*, 2015).

*AlkB* and *Nah* are two petroleum-degrading genes reported to be distributed in the soils of the Dagang Oil field, Tianjin, China. The *AlkB* gene having positive correlation with the hydrocarbon and alkanes concentration and *Nah* gene being negatively correlated with concentrations of total aromatic hydrocarbons (Liu *et al.*, 2015). A number of cyanobacteria species have also been identified in Ranchi district of Jharkhand state of Bharat (Rahul *et al.*, 2016) which can be used for the mitigation of oil spills when used in mixed cultures (Raghukumar *et al.*, 2001).

The current study aims to isolate oil degrading bacteria from soil and test its ability to degrade different types of oil samples. Detailed methodology of collection and screening of current bacteria are the highlights of current study.

## Materials and Methods:

**Sample collection:** From a nearby motor garage, oil soaked soil samples were collected from the depth of 2 inch and 4 inch form the surface and brought to laboratory. Serially diluted samples were cultured and screened for isolation of different types of bacteria.

**Screening of bacteria:** *P. aeruginosa* was isolated by using above method and furthered maintained on the Blood Agar Medium.

**Oil Samples:** Different oil samples that were obtained from local sources:

- Burned Engine oil
- Fresh Diesel
- Nurani oil
- Fresh vegetable oil

**Inoculation:** *P. aeruginosa* was inoculated in different percentages of oils samples (2%, 4%, 6%, 8% and 10%) in test tubes and a control which contained oil with only media (no culture) with final volume being 2 ml.

**Incubation:** The test tubes were incubated at 37°C and observed after 24 hours, 48 hours, 72 hours, 5 days, 10 days and 15 days. Consumption of oil layer was monitored and compared with control.

## Result and Discussions:

The bacteria obtained on the Blood Agar Medium was inoculated into the different types of oil samples, was found to have the ability to degrade not only diesel and Engine oil, but also vegetable oil and nurani oil that are used for household purposes.

Figure 1 shows the extent of degradation of Diesel at different concentrations (2%, 4%, 6%, 8% and 10%). The sub-part 'A' of every figure shows the level of oil at the time of inoculation and part 'B' shows the oil level on 15th day. Figure 2, 3 and 4 show the degradation of vegetable oil, nurani oil and engine oil respectively. After 15 days, the oil degradation is clearly observed.

Further, genetic level studies need to be done to identify the isolated bacteria and also identify and purify the gene(s) responsible for the degradation of hydrocarbons.

### 1. Diesel Degradation

Figure 1.1: Positive control (No bacteria

Figure 1.2: 2 % Oil concentration

Figure 1.3: 4 % Oil concentration

Figure 1.4: 6 % Oil concentration

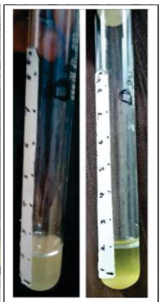

Figure 1.5: 8 % Oil concentration

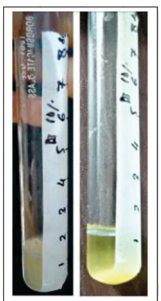

Figure 1.6: 10 % Oil concentration

### 2. Vegetable Oil Degradation

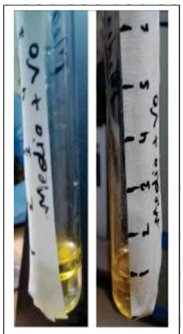

Figure 2.1: Positive control (No bacteria present)

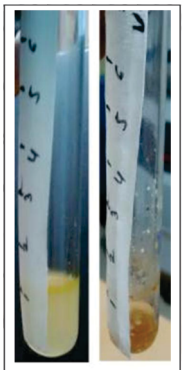

Figure 2.2: 2 % Oil concentration

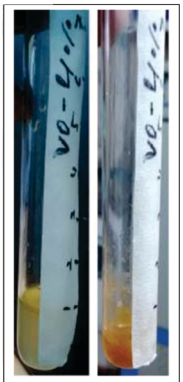

Figure 2.3: 4 % Oil concentration

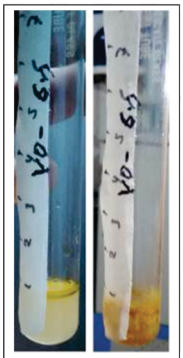

Figure 2.4: 6 % Oil concentration

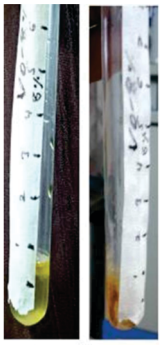

Figure 2.5: 8 % Oil concentration

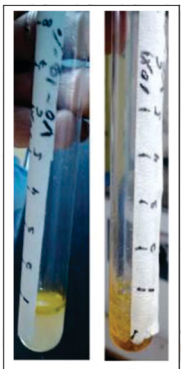

Figure 2.6: 10 % Oil concentration

### 3. Nurani Oil Degradation

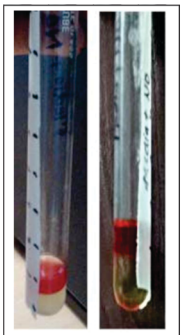

Figure 3.1: Positive control (No bacteria present)

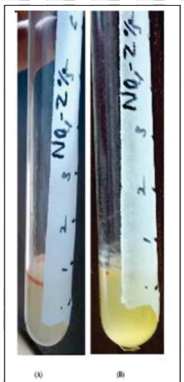

Figure 3.2: 2 % Oil concentration

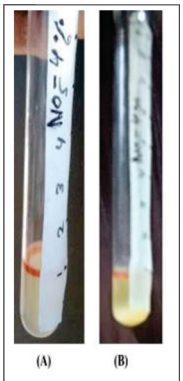

Figure 3.3: 4 % Oil concentration

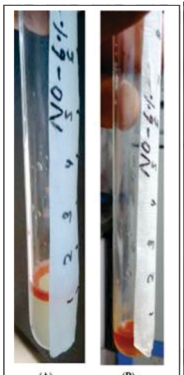

Figure 3.4: 6 % Oil concentration

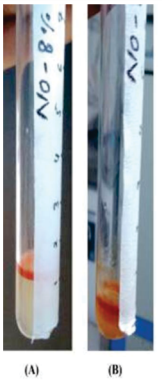

Figure 3.5: 8 % Oil concentration

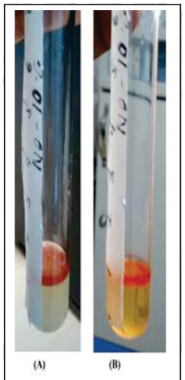

Figure 3.6: 10 % Oil concentration

4. Burned Engine

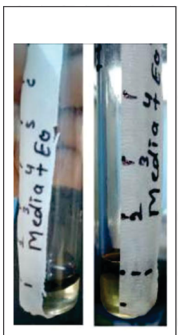

Figure 4.1: Positive control (No bacteria present)

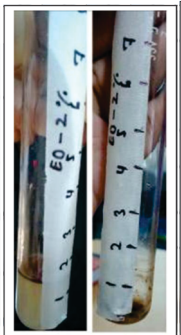

Figure 4.2: 2 % Oil concentration

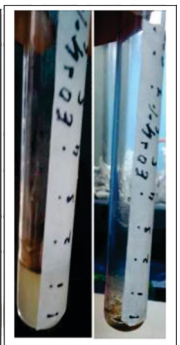

Figure 4.3: 4 % Oil concentration

Figure 4.4: 6 % Oil concentration

Figure 4.5: 8 % Oil concentration

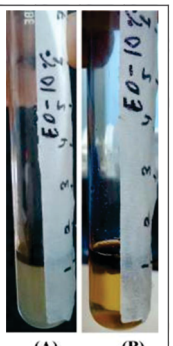

Figure 4.6: 10 % Oil concentration

## Conclusion:

Oil spills have devastating impact on all the living being that come into the range of the affected area. A number of oil degrading microbes are found in diverse habitats. These microbes can be helpful in rapid elimination of the hydrocarbons. This study aims at investigating the ability of the bacteria isolated from various sources to degrade various types of oils. The bacteria isolated from different sources were exposed to different oil samples. After duration of 15 days, degradation of oils was clearly visible in all the inoculated test tubes. Thus, it can be concluded that the bacteria isolated from different soil samples can successfully degrade any kind of oil and can be used bioremediation process after few more detailed studies.

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