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# EVALUATION OF OIL DEGRADATION POTENTIAL OF *BACILLUS- SUBTILIS.*

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**ABSTRACT**

The present study is carried out by isolation and characterization of oil degrading microbes from oil contaminated sites. In this work the *Bacillus* subtillis were isolated according to Bergey's manual and further used for oil degradation to check the production of biosurfactant. The Shake flask method and spread plate method were used for oil degradation. Further the optimization was carried out for best carbon, nitrogen sources and also for pH and temperature.

**Key words:** Bioremediation, Shake flask method, Spread plate method and optimization.

## INTRODUCTION

Petroleum hydrocarbon can be degraded by microorganisms such as bacteria, fungi, yeast, and microalgae. Numerous studies have been conducted on microbial consortia and enrichment and most bacterial petroleum hydrocarbon degraders have been isolated from heavily contaminated coastal areas.

Oil spill have been recognized as one of the most serious current particularly in industrialized and developing countries. Inevitable spillages, which occur during routine operations of oil products, refining or as a consequence of acute accidents, lead hydrocarbons to reach the water table before becoming immobilized in soil. They spread horizontally on the ground surface, leading loss of soil fertility and water holding capacity. Because of great number of oil contaminated sites requiring cleanup and high cost involved with conventional approaches for excavation and landfills, a need arises to develop new remediate technologies, such as bioremediation that uses microorganisms to detoxify environmental pollutants and transform into simpler less toxic compounds.

Oil spill are causing a major threat to the soil as well as water body. **Nwaogul,** *et al.,* 2008 reported the diesel oil spills on agriculture land generally reduces plant growth in diesel oil contaminated soils range from direct toxic effect on plants **Baker,** *et al*., 1982 and reduced germination **Udo,** *et al.,* 1975 to unsatisfactory soil conditions due to insufficient aeration of the soil because of the displacement of air from the space between the soil particles by diesel oil. Contamination of the soil by oil also causes it to lose its useful properties such as fertility, water-holding capacity, permeability and binding capacity. Contamination of groundwater is also a potential problem. The other significant impact is on surface water, mostly the nearby streams, which receive a lot untreated effluent form service stations containing oil and grease as well as non-biodegradable detergents **Moorthi,** *et al.,* 2008.

When an oil spill has occured, some section of the general public and some environmental pressure groups might say that the only acceptable oil spill response startegy is the total removal of the oil and complete restoration of the environment to the pre-spill condition.

Since this can never be met and some people always consider that any oil spill response is only a partial success.

Spilled oil has the potential to cause ecological effects, yet crude oil has been seeping into the sea for thousands of years at some locations around the world. These natural oil seeps have not caused major damage and the ecology of these areas has adapted to persistent and chronic oil pollution. Accidental spills of oil can deposit very large volumes into the sea over a short period of times and in a comparatively localised area. This can cause temporary ecological damage, although natural recovery will eventually occur. The physical effects of the oil spill, plus the less visible affects the some marine resources in a localised area.

The dead and dying seabirds covered in thick, sticky oil have become the 'icon' of oil pollution in the last decades.

The parameters typically measured in laboratory tests for bioremediation efficacy include enumeration of microbial populations, determinations of fate of hydrocarbon degradation. Undoubtedly, the most direct measure of bioremediation efficacy is the monitoring of hydrocarbon degradation or disappearance rates. Petroleum products such as petrol, engine oil, diesel and kerosene are used day to day in various forms at oil mechanic workshops. These products lead to hardening and change in colour of the solid which may have unseen and untold health hazard on the technicians, artisans and other co-workers. This study, thus was aimed to assess the unused oil biodegradation potential of selected bacterial strain under in vitro conditions.

Animals that rely on scent to find their babies or mothers fade away due to the strong scent of oil. This causes a baby to be rejected and abandoned, leaving the babies starve and eventually die. Oil can impair a bird's ability to fly, preventing it from foraging or escaping from predators.

The majority of birds affected by oil spills die without human intervention. Some studies have suggested that less than one percent of oil- soaked birds survive, even after cleaning although the survival rate can also exceed ninety percent, as in the case of Treasure oil spill.

**Factors of Bioremediation:**

The control and optimization of bioremediation processes is a complex system of many factors. These factors include: the existence of a microbial population capable of degrading the pollutants; the availability of contaminants to the microbial populations; the environment factors (type of soil, temperatures, pH, the presence of oxygen or other electron acceptors, and nutrients).

The bioremediation agents Inipol EAP22 and "Customblen" were shown to stimulate oil biodegradation on cobble beaches in Alaska after the *Exxon Valdez* incident **(Pritchard & Costa, 1991; Bragg *et al.* 1994).** This has led to the

suggestion that research in bioremediation now needs to be focused on operational aspects of the technology **(Swannell & Head, 1994).**

## METHODOLOGY

**Sample collection:**

The samples collected from different oil contaminated sites.

**Isolation of bacteria:**

The soil sample was serially diluted in normal saline solution and then spread on sterilized nutrient agar plates. The colonies were selected on the basis of their morphological parameters and pure cultures were prepared.

**Screening of oil degrading bacteria:**

The pure cultures were re streaked on sterilized minimal salt agar media supplemented with 5% crude oil and clear zones indicates the positive cultures.

**Media optimization:**

The media components were optimized on the basis of one factor at a time rule for enhancing the oil degradation potential and then the percentage degradation was calculated from the optimization.

**Calculation of oil degradation percentage of culture grgm 01.**

Similarly, as described above for GRGM 01, but here instead of inoculating MSM with culture GRGM 01inoculation with best culture were used. After 7 days % yield was calculated by formula:

**% Oil degradation =** (Volume of oil on zero days – Volume of oil on $7^{th}$ day x 100) / (Volume of oil on zero days)

**Strain identification:**

The cultures were identified by following the biochemical tests based on the Bergy's manual.

## RESULTS

**Soil sample collection:**

Two soil samples were collected from different places within Lucknow city.

**Table 1:** List of Soil samples collected from different areas.

| S. No. | Soil Type | Place |
|---|---|---|
| 1. | Dry Soil | Roadways Bus-stand Gomtinagar, Lucknow. |
| 2. | Wet Soil | Chhinhat workshop Gomtinagar, Lucknow. |


**Figure 1:** Soil Sample collected from different areas

**Isolation of oil degrading bacteria**

Bacteria from oil contaminated soil were isolated by serial dilution method and crowded plate method, mixed culture was obtained. These cultures were named as GRGM 01, GRGM 02, GRGM 03, GRGM 04, GRGM 05 and GRGM 06. These colonies were selected for further work.

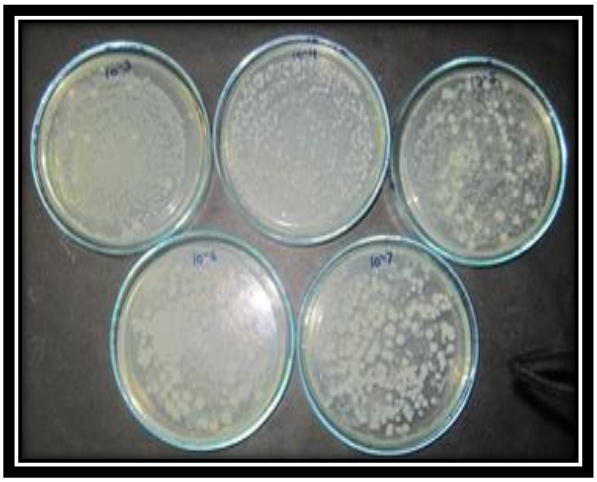

**Figure 2:** Spreading of were studied according to Bergey's Manual

**Colony morphology**

Colony morphology were studied according to Bergey's Manual

**Table 2:** colony morphology of selected culture

| Culture | Shape | Margin | Elevation | Pigmentation | Texture |
|---|---|---|---|---|---|
| GRGM01 | Irregular | Lobate | Convex | Yellowish | Rough |
| GRGM02 | Irregular | Discrete | Convex | White | Rough |
| GRGM 03 | Irregular | Entire | Convex | Creamy | Rough |
| GRGM 04 | Circular | Entire | Flat | Creamy | Smooth |
| GRGM 05 | Circular | Discrete | Raised | White | Smooth |

**Purification of obtained mixed culture**

Purification of culture was done by Quadrant streak plate method as shown below in figure 3.

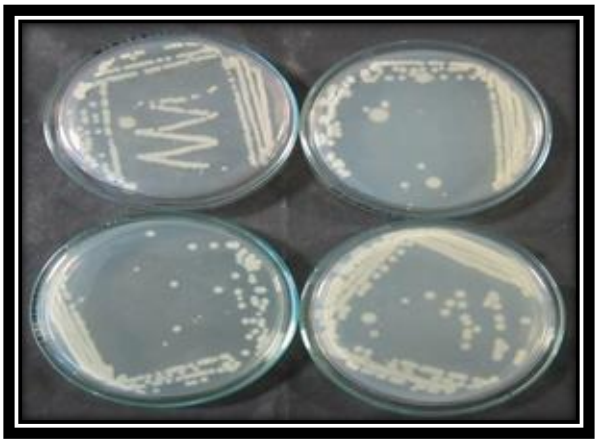

**Figure3:** Pure Culture

**SCREENING OF ISOLATES FOR OIL DEGRADATION POTENTIAL**

Screening of purified isolates for oil degradation was done by inoculating them in MSM medium supplemented with 10% engine oil and studying its protein profile. The data can be seen below in figures.

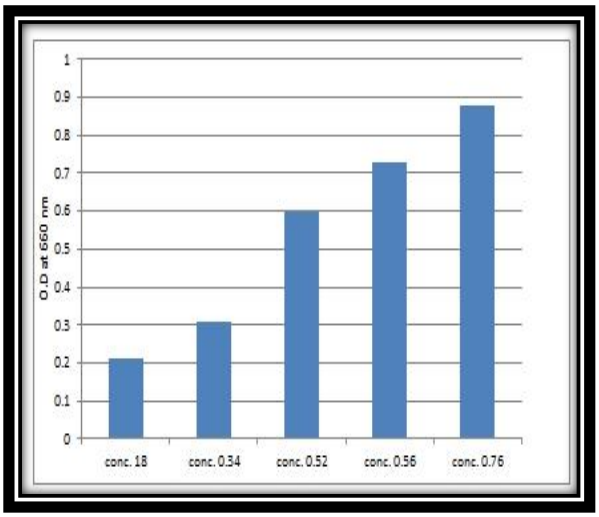


**Figure 4:** Graph showing protein profile of the GRGM 01

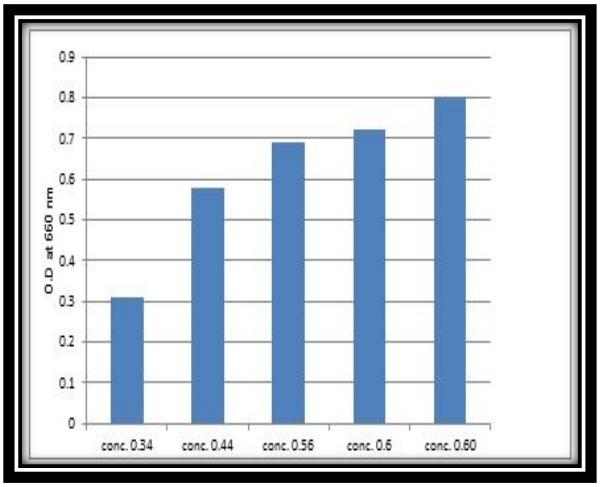


**Figure 5:** Graph showing protein profile of the GRGM 02

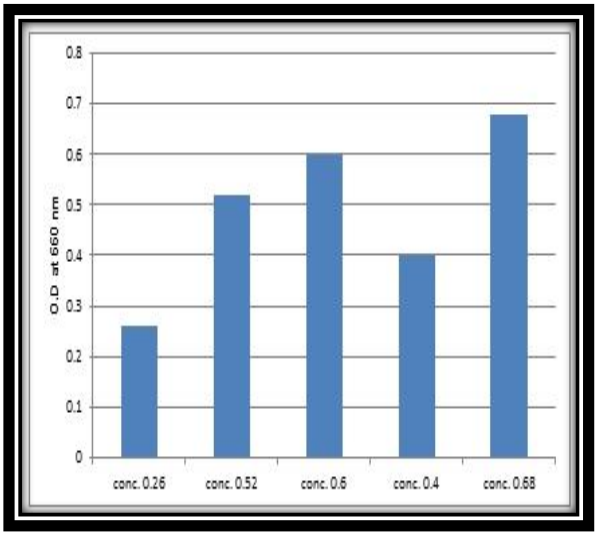


**Figure 6:** Graph showing protein profile of the GRGM 03

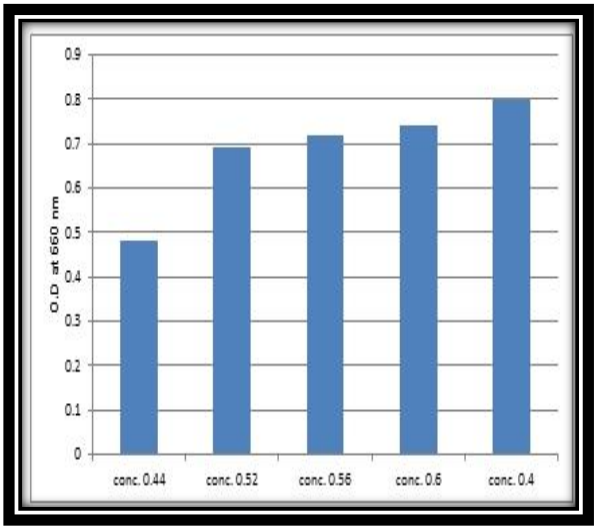


**Figure 7:** Graph showing protein profile of the GRGM 04

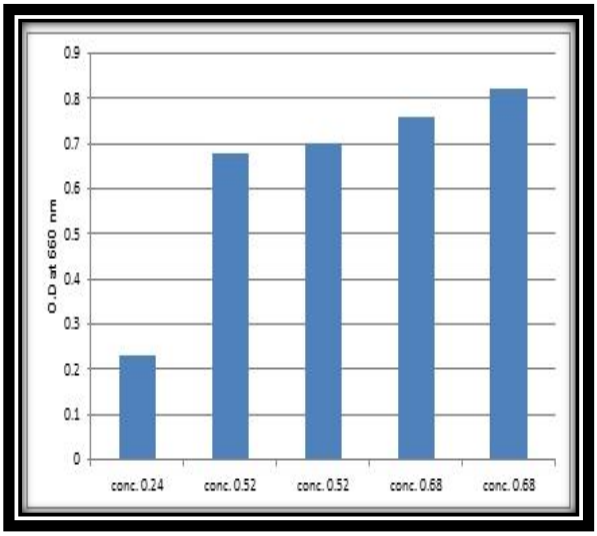


**Figure 8:** Graph showing protein profile of the GRGM 05

**Screening of broth supplemented with 10% oil**

**Figure 9 :**Flask showing culture GRGM 01, 02, 03, 04, and 05 with oil on 7$^{th}$ day.

**Identification of isolates showing maximum oil degradation potential (O.D.P)**

The selected culture GRGM 01 was identified by Bergey's Manual. Various Biochemical tests were performed.

**Table 16:** Staining & Biochemical Tests of GRVGM01.

| S. No. | Tests | Result |
|---|---|---|
| 1. | Gram Staining | Positive (*Bacillus*) |
| 2. | Endospore test | Positive |
| 3. | Catalase test | Positive |
| 4. | Mannitol test | Positive |
| 5. | VP test | Positive |

**Optimization of physiochemical factors optimum growth of isolate GRGM01**

**Table 17**: effect of temperature on GRVGM 01

| S. No. | Temperature | Growth | Remarks |
|---|---|---|---|
| 1. | 22°C | No Growth | - |
| 2. | 37°C | Growth Observed | ++ |
| 3. | 28°C | Growth Observed | +++ |
| 4. | 50°C | No Growth | - |

**Effect of pH on growth of GRGM 01**

The MSM media of culture was maintained at different pH as 4.0, 7.0 & 9.0 to check the effect of pH on the culture. The maximum growth was observed at pH 7.0 Here are the results.

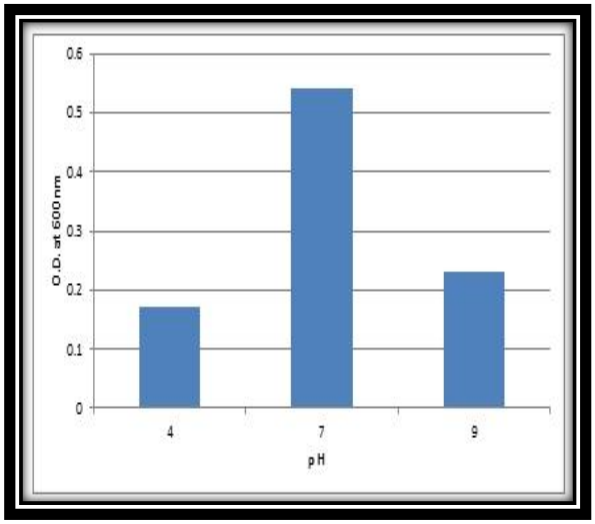


**Figure 15:** Graph showing effect of pH on GRVGM 01

**Effect of different nitrogen sources on O.D.P**

Different MSM media were made which differed from the core production media in Nitrogen sources like, $NH_4Cl$, Beef Extract, and Yeast Extract.

O.D at 600 nm of each of the above MSM media with different nitrogen sources was taken after 24 hours of inoculation to find out which support the maximum O.D.P

| NITROGEN SOURCE | O.D at 660 nm |
|---|---|
| $NH_4Cl$ | 0.44 |
| Beef Extract | 0.63 |
| Yeast Extract | 0.86 |

This Yeast Extract is the most suitable.

**Effect of different carbon sources on O.D.P**

Different MSM media were made which differed from the core production media in Carbon sources like, Glucose, Sucrose, and Maltose.

Protein profile of each of the above MSM media with different carbon sources was done by Lowry's method after 24 hours of inoculation to find out which supports the maximum O.D.P

| CARBON SOURCE | O.D at 660 nm |
|---|---|
| Glucose | 0.39 |
| Sucrose | 0.49 |
| Maltose | 0.62 |

Thus Maltose is most suitable.

**Calculation of percentage of oil degradation potential of best culture GRGM 01.**

The percentage of calculation of oil degradation of best culture was performed as all above

After 7 days 5ml oil was recoverd, oil degradation potential can be calculated from formula given below:

**% oil degradation =** (volume of oil on zero days - volume of oil on 7th days x 100) / (Volume of oil on zero days )

**% oil degradation =** (10–5 x 100) / 10

From the above equtation it eas shown that 50% oil was degraded .

## DISCUSSION

In this study the soil sample was collected from oil contaminated site because the capability of native bacterial population to mineralize crude oil contaminated hydrocarbons in crude oil contaminated site were confirmed before by many researchers **(Sepahi., 2008, Ojo., 2006).** Further microorganism was isolated by serial dilution agar plate techniques as previously done by (**Udeani, *et al*., 2009 & Khan, J.A and Rizvi, S.H.A., 2011)**. Purified culture was characterized for various staining and biochemical tests according to Bergey's manual.

The growth of the microorganism in Mineral salt media supplemented with engine oil was determined by protein estimation throughout incubation period that reflect the ability of isolated *Bacillus* species to degrade & utilize crude oil as carbon source. This technique has been used in several studies to determine the oil degradation potential of bacteria in crude oil. **(Sepahi & Mittal & Singh).**

## CONCLUSION

Oil spill are a major threat to the environment as they adversely affect the surrounding ecosystem. Bioremediation is the best way to treat the oil contamination. The microorganisms present at oil contaminated sites have ability to degrade toxic contaminants present in oils into nontoxic forms.

Several works are going on to isolate different types of microbial strain that have ability to degrade oil. In this study a *Bacillus* species was isolated and oil degradation studies were performed. Increase in turbidity & protein concentration during incubation period revealed that the isolated Bacillus species has ability to degrade and utilize crude oil as source of carbon and energy.

Future prospects of the present research work include application of the broth culture of the isolated oil degrading strains to oil contaminated soil and water bodies.

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