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# Bioremediation of Oil Contaminated Soil Using Bacterial Strain and Its Application

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**ABSTRACT**

Oil spill, which is a common problem around the world, could be tackled by bioremediation. Oil spill soil samples were collected from Chennai Petroleum Corporation Ltd. (CPCL). It was observed that 19mg/gm of oil spills was present in the soil. For eradicating this oil spills from soil, natural and synthetic media were used. Gingerly cake and Cotton seed cake as natural media and Nutrient broth as artificial medium was used. Organisms present in the sample were allowed to grow in all these media and was subjected for lipase activity. The specific activity of cotton seed cake for *Bacillus* species showed value of 260377.35U/mg and for *Pseudomonas* was 167058.82U/mg compared to other media. It was observed that Cotton seed cake media was best media and it is cheaper compared to other media. The gravity analysis report showed that initial value of 19mg/gm of oil spill soil was degraded to 5.7mg/gm in case of *Bacillus.* This *Bacillus* species had led to degradation of 13.3 mg/gm of oil spills.

**KEY WORDS:** *Bacillus*, Oil Spills, Gingely oil cake, *Pseudomonas,* Cotton seed cake.

## 1. INTRODUCTION

The impact of oil spill in aquatic and terrestrial environments is alarming. The oil spill contains both unsaturated and saturated hydrocarbons which are major cause of pollution. This problem is being tackled by physical and chemical means at present.[3] Microorganisms, which can utilise hydrocarbon as the sole source of carbon for their energy needs are found in nature. These microorganism could be good agents to degrade the oil spills. Although various methods such as using booms, skimmers, adsorbants etc. to remove the pills are in use, they are not effective.[4] The present study focussed at the following objectives namely, to isolate potent lipase producing organism for the degradation of oil spill sample, production of enzyme lipase using locally available cost effective substance, to determine the hydrocarbon degradation ability of bacteria isolated from oil spilled soil sample, to study the application of completely biodegraded oil spill soil sample.

## 2. MATERIALS AND METHODS

**Collection of soil sample:** 500g of contaminated spol with petroleum hydrocarbon was used for isolation of hydrocarbon utilizing organism collected from Chennai petroleum corporation Ldt. (CPCL), Chennai. Sub surface soil sample contaminated with petroleum hydrocarbon where collect in free soil sample bottles. The sample where label and stored at -4 $^0$C for further analysis.

**Collection of Substrate:** Substrate sample Cotton cake and Gingelly cake was collected from local store at Tambaram, Chennai.

**Media Preparation**

**Nutrient Broth:** 1.7g of nutrient broth was dissolved in 100ml distilled water. It was then autoclaved at 15lbs pressure for sterilization purpose.

**Cotton Broth:** 15g of cotton cake substrate was dissolved in water and then autoclaved at 15lbs. After autoclaving it was grinded with the help of morter and pistle. The filtered supernatant was used as cotton broth.

**Gingely Broth:** 15g of gingelly cake substrate was dissolved in water and then autoclaved at 15lbs. After autoclaving it was grinded with the help of morter and pestle. The filtered supernatant was used as gingely broth.

**Serial dilution:** The standard serial dilution plate technique was followed for isolating bacteria in Nutrient agar media.

One gram dried soil was suspended in 9ml of distilled water and mixed thoroughly. D dilutions of $10^{-3}$, $10^{-5}$, $10^{-7}$ of the suspension were applied on to petri plates to which 20ml of melted medium waere added to each plate. The plates were incubated at 37$^0$C for 24 hours. Enumeration of different isolates was carried out. Selected colonies of bacteria were transferred from mixed culture on to respective agar plates containing pure culture were stored at 4$^0$C united for the examinations.

**Spread plate method:** The spread plate technique was used to enumerate the aerobic microorganisms by counting the colonies after incubation.

**Biochemical test for identification of unknown species**

**Catalase test:** To demonstrate the presence of catalase in an organism catalase test is performed.

**Oxidase Test:** The enzyme oxidase that froms the part of electron transport system is possessed by some bacteria. The enzyme oxidises the reagent N N tetramethy l paraphenylene diamine dihydrochloride to a coloured product indophenols. When the growth of the organism is rubbed over the filter paper containing this reagent, a purple colour will be developed.

**Methyl Red and Voges Proskauer Test:** To find out whether an organism is MR/VP positive or not.

**Result and Interpretation**

Mr test: red - positive
yellow - negative
Vp test pink - positive
yellow/colourless - negative

**Citrate Utilization Test:** Certain organism can utilise citrate as sole carbon source and grow. During the growth acetate and other alkaline carbonates are produced. This reaction is shown by the changes in the colour of the indicator.

**Result and Interpretation**

Blue colour - Positive
Greeish or No change -Negative

**Indole Test:** To determine the ability of an organism to produce Indole.

**Qualitative Test:** The ability of four *Bacillus* to produce lipase was tested. Medium (Serra, 1957) containing Tween 80 as lipid substrate, at pH 8.0 was used for qualitative test of lipase. The lipolytic activity of each bacterial strain was determined by measuring the diameter of hydrolytic zones around each colony. The strain with the largest zone of hydrolysis was used for further study.

## 2. MATERIALS AND METHODS

The culture media were poured on petri plates uniformly and the inocula were spread on it uniformly. The plates were incubated up to 72 hours under optimal conditions (e.g. 28 °C).

Lipolytic microorganisms produce colonies which are surrounded by clear zones in the turbid culture medium on the plate.

**Enzymatic Assay of Lipase (Olive Oil as Substrate)**

**Principle**

Triglyceride + $H_2O$ Lipase > Diglyceride + Fatty Acid

**Conditions**

T = 37 $^0$C, pH = 7.7

**Method**

Titrimetric

**Protein Estimation by Lowry's method**

**Objective**

To determine the concentration of protein by Lowry's method.

## 3. RESULTS AND DISCUSSION

**Isolation of Bacterial from Contaminated Soil:** Bacteria were isolated from contaminated soil samples by serial dilution technique. Two types of bacteria namely, *Pseudomonas* and *Bacillus* sp. were identified, isolated and supernatant of those bacterial samples were poured into wells for checking lipase activity as shown in figure 1a 1b.

**Identification of organism using Biochemical assay:** Under these series of 6 biochemical test shown in Table 2, positive results were shown by bacterial species of *pseudomonas* and *bacillus*.

**Estimation of Specific Activity:** For Green culture (*Pseudomonas*), the specific activity of lipase enzyme is more in case of Cotton seed broth (167058.82 U/mg) is higher compared to Nutrient broth (132142.85 U/mg) and Gingelly cake (132258.06 U/mg) as shown in Table 3.

For White culture (*Bacillus*), the specific activity of lipase enzyme is more in case of Cotton seed Broth (260377.35 U/mg) is higher compared to Nutrient broth (217142.85 U/mg) and Gingelly cake (76056.33 U/mg) as shown in Table 4.

On observing both the Table 3 and 4, it is clear that specific activity of lipase enzyme is comparatively more in case of Cotton seed cake (Natural media) than that of Nutrient broth (Synthetic) and gingelly cake (Natural media).

**Gravity Analysis:**

**Initial Oil Spills Soil Gravity Analysis:** Initial gravity analysis report shows that raw soil contains oil spills was measured to be 19mg/gm of soil.

**Enzyme Treated Oil Spills Soil Gravity Analysis:** This soil showed the reduced gravity value as 5.7mg/gm of soil compared to that of untreated soil sample .This values shows the effectiveness of lipase enzyme in degradation of oil spills. Initial value was 19mg/gm and the final value was found to be 5.7mg/gm, this shows a reduced value of **13.3mg/gm** of soil .This reduction is entirely due to lipase activity.

**Application:**

**Table.1. Protein Estimation by Lowry's method**

| S.No | Particle | B | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Working BSA (ml) | - | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 | - | - | - | - | - | - |
| 2 | Working BSA conc. | - | 40 | 80 | 120 | 160 | 200 | - | - | - | - | - | - |
| 3 | Sample (ml) | - | - | - | - | - | - | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| 4 | Distilled Water (ml) | 1 | 0.8 | 0.6 | 0.4 | 0.2 | - | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |
| 5 | Reagent C (ml) | 5 | | | | | | | | | | | |
| 6 | Folin's (ml) | 0.5 | | | | | | | | | | | |
| 7 | Optical Density at $660_{nm}$ | 0 | 0.8 | 0.18 | 0.23 | 0.32 | 0.40 | 0.45 | 0.68 | 0.50 | 0.28 | 0.43 | 0.57 |

**Table.2. Biochemical Test**

| S.no | Biochemical test | Pseudomonas (Green culture) | Bacillus (White culture) |
|---|---|---|---|
| 1 | Catalase | + | + |
| 2 | Oxidase | - | - |
| 3 | Indole | - | - |
| 4 | MR | - | - |
| 5 | VP | + | - |
| 6 | Citrate | + | + |

**Table.3. Green Culture**

| Culture | Nutrient Broth | | | Cotton Seed Cake | | | Gingelly Cake | | |
|---|---|---|---|---|---|---|---|---|---|
| | Enzyme Activity | Protein | Specific Activity | Enzyme Activity | Protein | Specific Activity | Enzyme Activity | Protein | Specific Activity |
| | U/mL | Mg/mL | U/mg | U/mL | Mg/mL | U/mg | U/mL | Mg/mL | U/mg |
| Green Culture | 296000 | 2.4 | 132142.85 | 568000 | 3.4 | 167058.82 | 328000 | 2.48 | 132258.0 |

**Table.4. White Culture**

| Culture | Nutrient Broth | | | Cotton Seed Cake | | | Gingelly Cake | | |
|---|---|---|---|---|---|---|---|---|---|
| | Enzyme Activity | Protein | Specific Activity | Enzyme Activity | Protein | Specific Activity | Enzyme Activity | Protein | Specific Activity |
| | U/mL | Mg/mL | U/mg | U/mL | Mg/mL | U/mg | U/mL | Mg/mL | U/mg |
| White Culture | 304000 | 1.4 | 217142.85 | 552000 | 2.12 | 260377.35 | 216000 | 2.84 | 76056.33 |

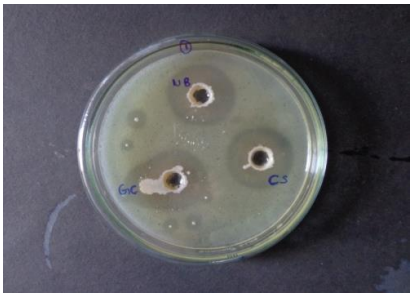

**Figure.1a. (Protein estimation)**

1- Green Culture (Pseudomonas)
NB- Nutrient media
G- Gingelly Cake broth

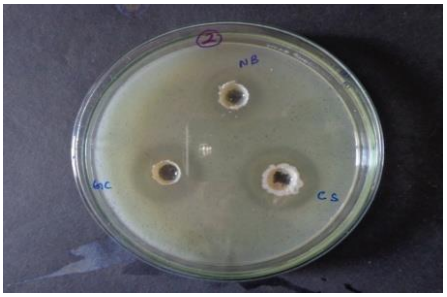

**Figure.1.b (Protein estimation)**

2- White Culture (Bacillus)
NB- Nutrient media
G- Gingelly Cake broth

**Figure.2. (After 3 days of seed rest in lipase enzyme treated soil, shows growth in the form of germinated seeds)**

## 4. CONCLUSION

*Bacillus* and *pseudomonas* was isolated from oil spill soil sample and this soil showed the reduced gravity value as 5.7mg/gm of soil compared to that of untreated soil sample .This values shows the effectiveness of lipase enzyme in degradation of oil spills. Initial value was 19mg/gm and the final value was found to be 5.7mg/gm, this shows a reduced value of 13.3mg/gm of soil

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