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# EVALUATION OF OIL DEGRADATION POTENTIAL OF *MICROCOCCUS VARIANS*

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**ABSTRACT :** In the present study three bacterial isolates namely MJS1101, MJS1102 and MJS1103 were isolated and purified from oil contaminated soil sample. Oil degradation potential of all the three isolates was evaluated by studying their growth and protein profile while they were inoculated in Minimal salt media supplemented with 5% used engine oil for a period of 10 days. The isolate MJS1102 showed maximum growth (Quantified by OD at 600nm and enumeration on Nutrient agar plates) throughout the incubation period and also concentration of protein in the flask containing MJS1102 was found to be maximum throughout the incubation period thereby by giving an indication that the isolate was able to utilize the hydrocarbons present in used engine oil as a source of carbon and energy. Percentage oil degradation by the isolate was also calculated and it was found to be 84.41%.

**Keywords:** Bioremediation, *Micrococcus varians,* Oil contaminated sites, Oil degradation.

## INTRODUCTION

We all contribute to oil pollution directly, by washing greasy hands and throwing away the water, dripping oil from a leaky engine sump, by spilling some fuel when refilling an outboard motor, and in many other ways. Everybody also contributes indirectly, by making use of the products of the oil industry, including petrol and diesel for motors, kerosene for lamps, electricity that has been generated using fuel, and many plastic and chemical products that we use every day (Carver and Carver, 1989).
Ships that travel with oil and other products derived from oil that are used as fuels, lubricants and other purposes, at times spill oil into the sea accidently. This becomes a thick layer on the surface of the water and then prevents oxygen getting into the water and carbon dioxide from coming out. Because most petroleum products are poisonous, the fish get contaminated with chemicals such as polychlorinated biphenyls (PCBs).

Routine and deliberate discharges, when tanks are flushed out with seawater, also add a lot of oil to the oceans. An oil spill has its worst effects when the oil slick encounters a shoreline. Oil in coastal waters kills tide pool life and harms birds and marine mammals by causing feathers and fur to lose their natural waterproof quality, which causes the animals to drown or die of cold, these animals can become sick or poisoned when they swallow the oil while preening, oil cannot dissolve in water and forms a thick sludge in the water. This suffocates fish, gets caught in the feathers of marine birds stopping them from flying and blocks light from photosynthetic aquatic plants, oil kills organisms and marine animals like fishes, crabs and other crustaceans, oil increase the underneath temperatures for both animals and plants, Planktons, larvae and small marine organisms, oil poisons algae, disrupts the major food chains and decrease the yield of edible Crustaceans, It also coats birds, impairing their flight or reducing the insulative property of their feathers, thus making the birds more vulnerable to cold, Oil on water surface also interferes with gaseous interchange at the sea surface thus, dissolved oxygen levels will thereby be lowered, additionally an oil spill can directly damage boats and gears used for catching or cultivating marine fishes (Obih Uchenna).

As per the estimates of United Nations, 1.3 billion barrels of oil are spilled annually into the, Persian Gulf and about 285 million gallons are spilled into oceans every year. Oil spills make up about 12% of the oil that enters the ocean. The rest come from shipping travel, drains and dumping. However, what makes tanker spills so destructive is the sheer quantity of oil they release at once. In other words, the concentration of oil they produce in one very localized part of the marine environment (Chris Woodford, 2011).

The above said ill effects of oil pollution have been a major threat to the environment and humans also, and that is the reason why a large number of remediation methods have been adopted during the previous decades including Dissolution, Emulsification, Oxidation and destruction, Sedimentation, Aggregation, Self-purification, In-situ burning but there has been one or more drawbacks with all of them (Stanislav Patin).

The bioremediation technique which is the only technique without any drawbacks is the first choice now for any researcher some of the advantages over other oil pollution remediation techniques being Low energy input, Moderate capital investment, Low operating cost, Environmentally safe, Minimum requirement of space and equipment, Can be done at site, More acceptable to public / community compared to conventional technologies. **(**Balba *et al*., 1998).

Taking into consideration the advantages of bioremediation over tradional methods the present study was started with an objective evaluate the micro flora inhabiting oil contaminated sites for their oil degradation potential.

## MATERIALS AND METHODS

Soil sample was collected from oil contaminated sites at Waheed Servicing Centre, HAL, Lucknow. Soil was collected randomly 5-10 cm beneath the surface using a sterile spatula and were packed in sterile polybags and transferred to the laboratory.

Used engine oil was collected from Bharat automobiles, Azad Market, Lucknow. When collected oil was Blackish Brown in colour and pH was 9.54.

Bacterial species were isolated from the collected soil sample by serial dilution agar plating method wherein the soil sample was diluted upto $10^{-5}$ dilution, and the diluted soil samples were spread on sterile Nutrient agar plates. The inoculated petri-plates were incubated at 37ºC for 24 hours. Mixed colonies were obtained in petri-plates after incubation, three different colonies differentiated on the basis of colony morphology namely MJS1101, MJS1102 and MJS1103 were picked and purified by quadrant streaking on sterile NA plates. The purity of cultures was cross checked by gram staining procedure.

Oil degradation potential of purified cultures was evaluated in Minimal salt media supplemented with 5% V/V used engine oil, media with oil was autoclaved at 15 psi for 20 minutes. Cooled media was inoculated with 1 ml of 24 hours old grown culture of respective pure cultures. The inoculated flasks were incubated at 37ºC for 10 days at 120 rpm in a shaking incubator. Oil degradation potential was quantified based of the growth and protein profile of the isolates throughout the incubation period. Growth profile included enumeration of the isolates by reading absorbance at 600 nm and also by spreading the cultures on NA plates. Protein profile included the estimation of protein throughout the incubation period. A good and increasing growth and protein profile was indicative of the oil degradation potential of the isolates as the media used *i.e* MSM was having very less amount of carbon source and if the isolate is able to grow in MSM supplemented with used engine oil for 10 days it indicates that the isolate is able to utilize the used engine oil as a source of carbon and energy.

The isolate MJS1102 which showed maximum oil degradation potential during studies was selected for further studies, and percentage oil degradation by isolate MJS1102 was calculated by the formula given by (Jirasripongpun, 2002) wherein 50 ml of MSM pH 7 supplemented with 5% of used engine oil was prepared, and autoclaved at 15 psi for 20 minutes. Media was inoculated using 500 µl of the isolate MJS1102 and incubated at 37ºC for 7 days. The amount of oil in the flask on zero day was weighed and the oil left after seven days incubation period was also weighed, the formula given below was used in order to calculate the percentage oil degradation. A control flask was also maintained without inoculation.

$$\% \text{ Oil Degradation} = \frac{[\text{ Weight of oil on zero day} - \text{Weight of oil after 7 days }]}{\text{Weight of oil on zero day}} \times 100$$

(Jirasripongpun, 2002)

## RESULTS

Bacterial species from oil contaminated sites were isolated by serial dilution agar plating method and mixed colonies were obtained, three different colonies (MJS110, MJS1102 and MJS1103) differentiated on the basis of colony morphology were purified by quadrant streaking and their purity was crosschecked by Gram's staining procedure.

The purified isolates were evaluated for their oil degradation potential in MSM supplemented with 5% used engine oil, oil degradation was quantified by studying the growth and protein profile throughout incubation period (10 days). Figure 1 below shows the growth profile of all the three isolates studied by taking the absorbance readings of the flask containing the respective isolates in MSM supplemented with 5% used engine oil. Table 1 below shows enumeration of the isolates in Nutrient agar paltes. Figure 2 below shows the standard graph prepared for protein estimation by Bradfrod's method. Figure 3 shows the protein profile of all the three isolates studied by estimating the concentration of protein by Bradford's method in the flask containing the respective isolates in MSM supplemented with used engine oil.

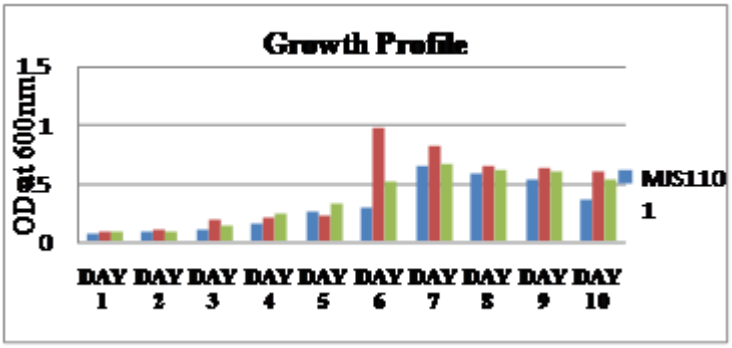


**Figure 1: Growth Profile.**

**Table1: Enumeration of Isolates on NA Plate.**

| DAYS | MJS1101 | MJS1102 | MJS1103 |
|---|---|---|---|
| Day 1 | Lawn | 124 colonies | Lawn |
| Day 2 | Lawn | Lawn | Lawn |
| Day 3 | Lawn | Lawn | Lawn |

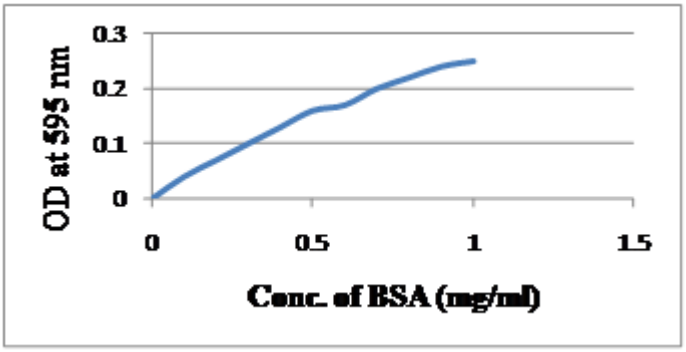


**Figure 2: Standard Graph for Bradford's Assay.**

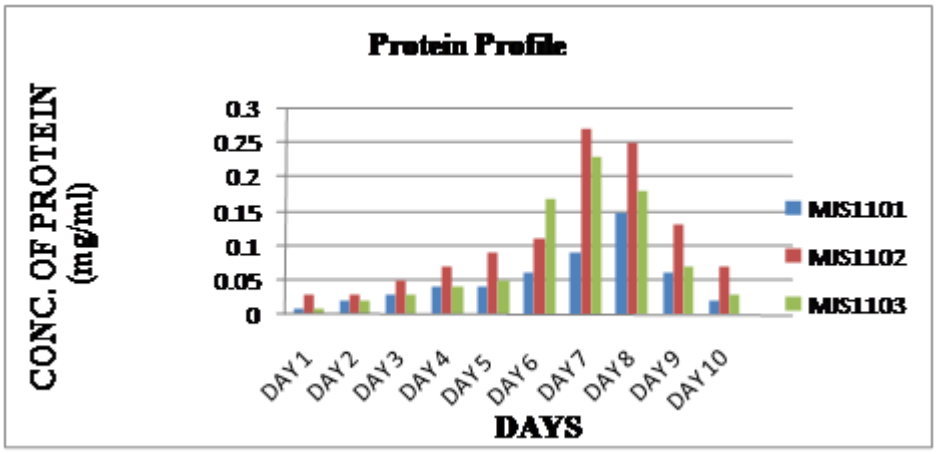


**Figure 3: Protein Profile.**

**Table 2** below shows the results of staining and various biochemical activities of the isolate MJS1102 showing maximum oil degradation potential. By comparing these results with Bergey's manual (Aneja., 2003) the isolate MJS1102 was tentatively identified as ***Micrococcus varians***.

**Table 2: Staining and Biochemical Activity of MJS 1102**

| S. No. | TEST | RESULT |
|---|---|---|
| **1** | GRAM STAINING | +ve, Monococcus |
| **2** | CATALASE TEST | +ve |
| **3** | GLUCOSE TEST | +ve |
| **4** | MANNITOL TEST | -ve |
| **5** | OXIDASE TEST | +ve |

The culture showing maximum oil degradation potential was used further to calculate the percentage oil degradation by the formula given by Jirasripongpun, 2002, **Table 3** below shows that oil was degraded upto 84.41%.

**Table 3: Percentage Oil Degradation.**

| S.NO | CULTURE | WEIGHT OF OIL ON ZERO DAY | WT OF OIL ON 7TH DAY | % OF OIL DEGRADATION |
|---|---|---|---|---|
| 1. | CONTROL | 1.963 | 1.963 | 0% |
| 2. | MJAN1102 | 1.963 | 0.306 | 84.41% |

## DISCUSSION

Soil sample was collected from two oil contaminated sites as done earlier by (Ojo, 2006; Okah, 2003; Emtiazi, *et al*., 2005; Khan and Rizvi, 2001). Further microorganism was isolated by serial dilution agar plating method as done previously by (Udeani *et al*., 2009). Cultures were purified by streaking techniques and the purity was cross checked by Gram staining procedure.

Purified cultures were screened for oil degradation potential in MSM (Minimal Salt Media) supplemented with 5% used engine oil on the basis of growth and protein profile throughout incubation period as done earlier by Sepahi *et al*., 2008.

Further, culture showing maximum oil degradation potential was characterized for the various staining and biochemical activities and was compared with Bergey's manual as done earlier by (Udeani *et al*., 2009) and was identified tentatively as *Micrococcus varians*. Few studies (Ijah and Antai, 2002; Ekpo and Udofia 2008) have been reported on the roles of *Micrococcus varians* in hydrocarbon bioremediation.

The isolate showed an oil degradation of **84.41%** after 7 days of incubation period. This is one of the few reports on this method of quantifying oil degradation potential.

## CONCLUSION

Based on the above study it can be concluded that *Micrococcus varians* can be a potent source for the remediation of oil contaminated sites and can act as a boon for the environment.

Future prospects of the current research includes the further enhancement in the oil degradation potential by using elicitors and also the evaluation of oil degradation potential of the isolate directly in oil contaminated soil and water bodies.

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