BIODEGRADATION OF CRUDE OIL BY GRAVIMETRIC ANALYSIS

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ABSTRACT

The widespread problem caused due to petroleum products, is their discharge and accidental spillage in the environment proving to be hazardous to the surroundings as well as life forms. Thus remediation of these hydrocarbons by natural decontamination process is of utmost importance. Bioremediation is a non-invasive and cost effective technique for the clean up of these petroleum hydrocarbons. The present study investigated about the isolation of bacteria from crude oil contaminated site (CPCL) and gravimetric analysis of degradation in which two bacterial isolates showed their maximum efficacy. Among ten bacterial isolates S10 showed maximum degrading efficacy (88.75%) followed by S2 that showed (87.41%.) degrading efficacy. Isolate S2 was identified as Bacillus subtilis and S10 was identified as Pseudomonas aeruginosa.

Keyword: Biodegradation, B.Subtilis, P.Aeruginosa, Gravimetry, Bioremediation

I. INTRODUCTION

Pollution is the entry of contaminants in the natural environment; it leads to cause instability, harm or discomfort to the ecosystem. Soil pollution is caused by the presence of xenobiotic chemicals or other sources in the natural soil environment. These occur when chemicals are released by spill or underground leakage. The most significant soil contaminants are hydrocarbons, heavy metals, herbicides and pesticides etc. All petroleum products are originated from crude oil whose major constituents are hydrocarbons .(Shigeaki etal,1999).Bacteria have long been considered as one of the predominant hydrocarbon degrading agents found in the environment, which are free living and ubiquitous (Dasgupta& R. Ghosh, 2013). The biodegradation of crude oil by microorganisms is one of the primary ways to remove crude oil from contaminated area. It has been studied that bacterium that grows in oil contaminated soil are much capable of degrading oil when compare with those bacteria which are found on non-contaminated soil. The presence of microorganisms with the appropriate metabolic capabilities is the most important requirement for oil spill bioremediation (Venosa&. Zhu, 2003). A large number of Pseudomonas strains capable of degrading PAHs have been isolated from soil and aquifers (Johnson et al ,1996; Kiyohara etal, 1992). Microorganisms have enzyme systems to degrade and utilize diesel oil as a source of carbon and energy (Antai, 1990). Mechanical method to reduce hydrocarbon pollution is expensive and time consuming. The type analysis provides the relative composition, the total amounts has to be determined by another method, called gravimetric method. So, the present study was designed to gravimetric analysis of crude oil degradation by bacteria.

II. MATERIALS & METHOD

Soil samples were taken from Chennai Petroleum Corporation Limited (Manali), and used for the isolation of bacterial cultures on nutrient agar. A total of ten bacterial isolates were isolated. The isolates were purified by streaking on agar plates and picking single colonies. The isolates were examined for various morphological and biochemical characteristics as per Bergey's Manual of determinative Bacteriology. The culture was maintained on nutrient agar medium at 4°C.

III. ISOLATION AND SCREENING OF CRUDE OIL DEGRADING BACTERIA

The isolation of bacteria from oil contaminated soil samples were performed by the following method as described by Latha and Kalaivani, 2012.

0.5 g of each oil spill contaminated soil samples were taken in 250 ml Erlenmeyer flask containing 50 ml of sterilized distilled water and 5 ml of oil (Diesel) as sole carbon source. The contents were mixed properly and incubated at 37°C on shaker at 160 rpm for 15 days. The incubated samples were subjected to serial dilution and serially diluted samples were plated on nutrient agar using spread plate method. The spread plates were then incubated at 37°C for 24 hours for the isolation of bacteria that has the ability to degrade oil spill. The bacterial colonies from the spread plate were subjected to quadrant streaking for single colony isolation. The individual bacterial colonies were streaked (backward and forward across the plate) onto respective agar plates and incubated at 37°C for 24hrs.

IV. GRAVIMETRIC ANALYSIS OF OIL

One gram of the soil was taken from each sample site. Petroleum ether and acetone were taken in the ratio 1:1 and was mixed with the soil sample in a separating funnel. The mixture was shaken for about 45 minutes and then was left undisturbed for about 10 minutes. The upper solvent along with oil was separated from the lower soil extract. The solvent with the oil layer was then kept in the hot air oven at 50º C until the solvent gets evaporated. After the complete evaporation, the oil residue obtained was weighed and taken as the gravimetric value for further calculation. Analysis of soil before and after treatment was done using this Gravimetric method (Saxena, 1990). The percentage of oil degraded was determined from the following formula:

$$\text{Percentage of oil degradation} = \frac{\text{Amount of crude oil degraded}}{\text{Amount of crude oil added in the media}} \times 100$$

Amount of crude oil degraded =(Weight of crude oil added in the media)- (Weight of residual crude oil)

Weight of Residual crude oil= (Weight of beaker containing extracted crude oil) – (Weight of empty beaker)

V. IDENTIFICATION OF THE ISOLATES

The colony characteristics and cellular morphology of the isolated, their pigmentation, staining reactions, physiological and biochemical characteristics were examined by standard methods and the isolates were identified.

VI. RESULT

A total of 10 hydrocarbon utilizing micro organism were isolated from the contaminated soil after enrichment process. Hence these 10 isolated designated S1 to S10 were selected for further screening of biodegradation rates. Among the 10 isolates, S2 and S10 more degrading efficacy than the others.S10 showed maximum degradaing efficacy (88.75%) followed by isolate S2 (87.41%.) degradation (Table 1). Hence, these two isolates S2 and S10 that showed the most efficient degradation than the other bacterial isolates (Fig : 1) .

Fig 1: Gravimetric Analysis of Crude Oil

The reduced optical density and increased colony forming unit, strains were used for secondary screening (Fig : 2). Among this crude oil degrading isolates were further checked for potential degradation ability by using gravimetric method. Only two bacterial isolates have the potency to degrade the crude oil.

Fig 2: Culture Preparation in Rotary Shaker

Based on various morphological, physiological and biochemical characterization, isolate S2 was identified as Bacillus subtilis and S10 as Pseudomonas aeruginosa, the results presented in (Table 2). Colony Morphology on nutrient agar plate, B. subtilis showed Creamy, big spreading, opaque and finely wrinkled. In P. aeruginosa showed large, pale yellow, convex, opaque circular colonies. (Table3).

Table: 1 Biodegradation of the Two Bacterial Isolates

Bacterial isolates	Oil degradation %
S2	87.41
S10	88.75

Table 2: Gram Stain and Biochemical Test Results

Biochemical Test	*Pseudomonas sp.*	*Bacillus sp.*
Gram staining	Gram Negative, Rod	Gram Positive, Rod
Catalase	Positive	Positive
Oxidase	Positive	Positive
Indole	Negative	Negative
Methyl Red	Negative	Negative
Voges proskauer	Negative	Positive
Citrate	Positive	Negative
Urease	Negative	Negative
Nitrate reduction	Negative	Positive

Table 3: Morphology of Bacterial Isolates in Agar Plates

Bacterial isolate	Shape	Elevation	Colour	Opacity
S2	Circular	Convex	Creamy	Opaque
S10	Circular	Convex	Pale yellow	Opaque

VII. CONCLUSION

In this study the isolation and identification of hydrocarbon degrading microbes from the crude oil collected from CPCL. P.aeruginosa was identified as the efficient degrader among the various bacterial isolates isolated which can be applied towards oil discharge and spill treatment. However, further scale-up studies as applicable need to be carried out in increasing the degrading ability and stability of the crude oil degrading isolate and its usage as a possible commercial strain.

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