

Isolation and Enrichment of Microbes for Degradation of Diesel Oil

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ABSTRACT

Oil refinery proves to be chief source of environmental pollution, which leads to human health hazards and damage soil surroundings. The present investigation aims to isolate petroleum tolerant and degrading microbes from oil contaminated soil of oil refinery, which later be used in environmental biotechnology as remediating mechanisms. Bushnell Haas Media (BHM) supplemented with diesel oil as carbon and energy source, used for isolation of oil degrading bacteria. The degraders were analyzed by streak plate method and bacteria were isolated by the selective enrichment culture plate technique and the biodegradation potential was determined.

Keywords: Environmental pollution, Petroleum, Microbes, Enrichment and Biodegradation

I. INTRODUCTION

Petroleum based products are the principle source of energy for day to day life but petroleum hydrocarbons is considered as toxic environmental pollutants [1]. Environmental contamination through petroleum products resulting from oil spillage during transportation of tankers, various industrial processes, and leakage in underground storage tanks damages the ecosystems [2]. Diesel oil is considered as one of the major constituent of crude oil which serve as essential fuel for vehicles and generators now a days [3].

Oil spills tend to leach down into the space between soil particles leads to insufficient aeration makes the agricultural land contaminated, however, reduces plant growth [4]. Hydrocarbons present in diesel oil once exposed to environment, persist in the ecosystem due to their hydrophobic and less volatility nature [5]. These hydrocarbons poses serious threats to human health due to mutagenic, carcinogenic and immunotoxic behaviour [6].

Microorganisms are capable of metabolizing hydrocarbons which are present in diesel oil into nontoxic biodegradable products as the sole source of carbon and energy [7]. Bioremediation through microbial degradation was considered as versatile, economic and environment friendly approach [8]. These microorganisms have catabolic genes which are able to synthesize metabolizing enzymes involved in hydrocarbon degradation [9]. A number of bacteria exhibits potential abilities in utilization of hydrocarbon substrates [10].

The adaptability and growing conditions of the microorganisms were analysed by enrichment culture processes. The selection of these microbes were detect by assessing the maximum specific growth rate as well as maximum final cell concentration [2]. The present investigation deals with the isolation of potential microbes capable of degrading diesel oil with respect to detoxification of environment.

II. METHODS AND MATERIAL

A. Sample Collection

Soil sample was collected from nearby sites around the oil refinery (Mathura). These samples were collected using trowel from 0 to 20 cm below the topsoil surface. The soil sample was brought to laboratory, air-dried, sieved through 200 mesh sieve (in order to remove plants or other waste residues), stored in polybags and kept in refrigerator till further analysis.

B. Isolation and Enrichment of Microbes

1gm of petroleum contaminated soil sample was suspended into 10 ml of normal saline was serially diluted to 1×10^7 times. 1ml of diluted sample was taken as inoculum in 100ml of Bushnell Hass broth [11] with 1ml of diesel oil. The flask then incubated in orbital shaker incubator at 37°C at 180rpm for 7, 14, 21, 28, 35 days. After individual cycles of incubations, bacterial colonies were sub cultured by streak plate method onto bushnell hass agar plates. Bacterial strains were isolated by selective enrichment culture plate technique.

C. Enhancement of diesel oil degradation capacity

To enhance the diesel oil degradation ability of microbes, they were transferred to the BH media containing increasing concentration of diesel oil.

D. Determination of diesel oil degradation

Bacterial growth rate was calculated by measuring the optical density of the flask's content. The turbidity obtained in the flask is directly proportional to the population of the bacteria involved in diesel oil degradation. The absorbance was measured in 7 days interval using Elico SL-159 model UV visible spectrophotometer at 600nm [12].

So, total three soil samples was collected around the sites near oil refinery.

B. Isolation and enrichment of microbes: About five microbial isolates was isolated from collected soil sample were checked for growth in 7 days interval from 7th to 35th days. Bacterial population in the enriched broth with diesel oil was the clear evidence for the hydrocarbon utilization in the flask (Figure 1) [15].

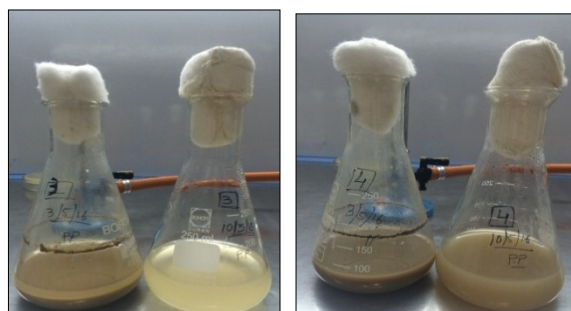


Figure 1. Flask containing broth after 7th - 35th day of incubation.

C. Enhancement of diesel oil degradation capacity:

Number of bacteria were found on the culture plate was sub cultured and streaked (with diesel oil) in a search of single isolated bacterial colony (Figure 2).

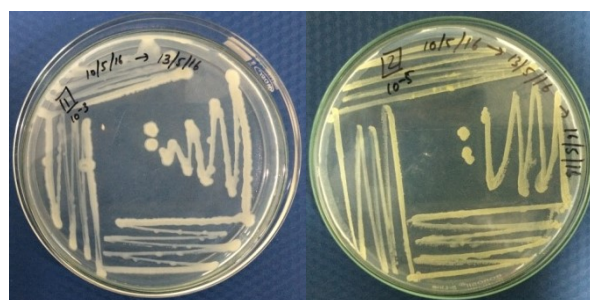


Figure 2. Streaked bacterial colonies on bushnell hass media with diesel oil.

D. Determination of diesel oil degradation

Selected bacterial strains when compared with control sample which was depriving of inoculum shows high amount to turbidity at 35th day of incubation. Flask containing the growth shown in fig 3. The ODs taken at 600 nm in the 7 day interval

III. RESULTS AND DISCUSSION

A. Sample Collection:

Oil industries are very prominent to contaminate the soils in the environment [13]. Bacterial population have the tendency to mineralize hydrocarbons present in diesel oil from various oil contaminated sites [14].

from 7th to 35th day of incubation were shown in Table 1.



Figure 3, Biodegradation potential of isolated hydrocarbon degrading bacteria.

The least turbidity were observed in control flask and maximum OD were 35th day of isolate. Similarly growth rate of these bacterial strains were plotted in Figure 4.

TABLE 1. OPTICAL DENSITY TAKEN AT 600NM

Sample	7 Days	14 Days	21 Days	28 Days	35 Days
Control	0.034	0.003	0.055	0.058	0.013
Isolate	0.598	1.153	1.069	1.123	1.597

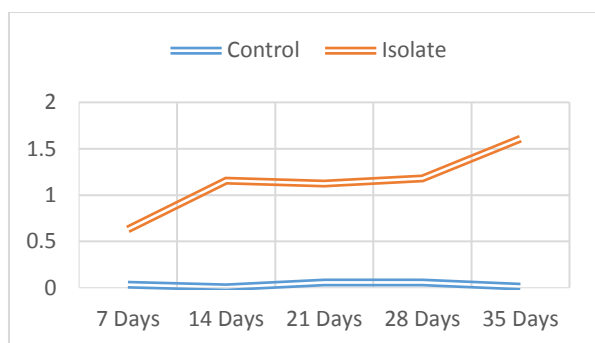


Figure 4. Graph showing biodegradation of bacterial isolate taken from 7th to 35th days of incubation.

IV. CONCLUSION

The results obtained in this study indicate that the salt of the Bushnell hass medium act as carbon and energy sources for the microbial growth. The degrading efficiency of isolated organisms was studied in BH

medium supplemented with diesel oil. It has also been observed that some microorganisms are more abundant in areas of high concentration of hydrocarbons. These microflora are actively oxidizing the hydrocarbons and this is considered as another source of carbon for use in the ecosystem. The experimental set up was kept for 35 days of incubation. The degradation was also checked spectrophotometrically at an OD of 620nm; where in the OD reading was found to fluctuate from increase to decrease and then decrease in all the causes till the end of incubation period. This confirms that all the isolates were capable of degrading diesel.

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