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ISOLATION AND ENRICHMENT OF MICROBES FOR BIODEGRADATION OF 4T ENGINE OIL

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Today environment change drastically due to human activity. Out of all the ecosystems terrestrial and marine ecosystems are affected majorly. Petroleum contaminated soil samples were collected Oil Garage station of Mumbai (Coastal area). Normal soil and Petroleum contaminated soil samples were physico-chemically characterized. Total Petroleum Hydrocarbons were separated by Colum Chromatography and it was analyzed by Gas Chromatography and Mass spectroscopy. Microorganisms were isolated by enrichment culture technique. 1%v/v 4T engine oil was used as a carbons source. These petroleum hydrocarbons degrading microorganisms were morphologically and biochemically also characterized.

Keywords: Colum Chromatography; Contaminated Soil; Enrichment culture Technique; Ecosystem.

Introduction

As the population is increasing day by day simultaneously the pollution in environment is also increasing proportionally. Due to fuel, shipping accident large amount to Poly aromatic hydrocarbons are entering into environment. These poly aromatic hydrocarbons are potential immuno toxic and carcinogenic.

Aromatic Hydrocarbons in the environment mostly originate from anthropogenic source like mineral oil spill or gas works plants and to a minor extent, from biological production in anoxic sediments. Numerous aerobic bacteria have been isolated that can breakdown aromatic hydrocarbons as carbon and energy sources. The bacterial degradation pathways of anthracene and phenanthrene have been elucidated[1].

Potential Sources of PAHs	References
Fossil fuels refining	2
Tobacco smoke	3
Air	4,5
Surface Water, Ground Water, and Road runoff	6
dispersed from the atmosphere to vegetation	7

PAHs present as natural constituents in fossils fuels, are formed during the incomplete combustion of fuel. The following are the possible sources for PAHs present in environment.

In the present investigation, main aim was to investigate the various physicochemical properties of soil which directly or indirectly influence the biodegradation potential of microorganisms.

Material and Methods

Normal soil sample and petroleum contaminated soil samples were collected from oil garage station of Mumbai. These soil samples were kept in sterilized poly bag. These soil samples were physically and chemically characterized.

Various properties like: PH of soil, Moisture content, Water holding capacity, carbonate and bi carbonate estimation, presence of heavy metal and many other parameters were studied. For chemical analysis Colum chromatography was performed for the separation of oil from soil. Silica gel of 60-120 mesh size was active and Hexane (Petroleum fraction) was used for aliphatic fraction while Toluene was used for aromatic fraction.

Result and Discussion

Physico chemical analysis of soil: different properties of soil were analyzed which are tabulated in Table 1.

Table 1. Physicochemical properties of petroleum contaminated soil

S No.	Parameters	Petroleum contaminated soil 1	Petroleum contaminated soil 2
1.	pH	8.25	8.10
2.	Bulk density	0.76	1.21
3.	Moisture content	12.16%	16.23%
4.	Water holding capacity	57.4	60.3
5.	Available phosphorous	-ve	-ve
6.	Calcium chloride	+ve	+ve
7.	Chloride	-ve	-ve
8.	Carbonate and Bicarbonate	+ve	+ve
9.	Organic matter	34.8%	28.8%
10.	Cu	+ve	-ve
11.	Fe	-ve	+ve
12.	Zn	+ve	-ve
13.	Cr	-ve	+ve
14.	Lead	-ve	-ve
15.	As	+ve	-ve

*Isolation of Microorganisms***:** CFU in PCS-1 and PCS-2 were shown in Table 2.

*Biochemical Characterization***:** From the Table 3 various biochemical tests were analyzed for isolated microorganisms.

Microbial Growth at 620nm: For Biodegradation determination OD at 620nm was taken and results are shown in Figure No.1.

Physico-chemical properties of soil play a vital role in biodegradation of petroleum compound. Environmental conditions are among the most important limiting factors for optimum results from

Table 2. Isolation and cell count of microorganisms

Samples	Enrichment Substrate	Cell Count
PCS	Original sample	0.7×10^{9}
	2nd Enrichment	4.3×10^{10}
	3rd Enrichment	6.2×10^{10}

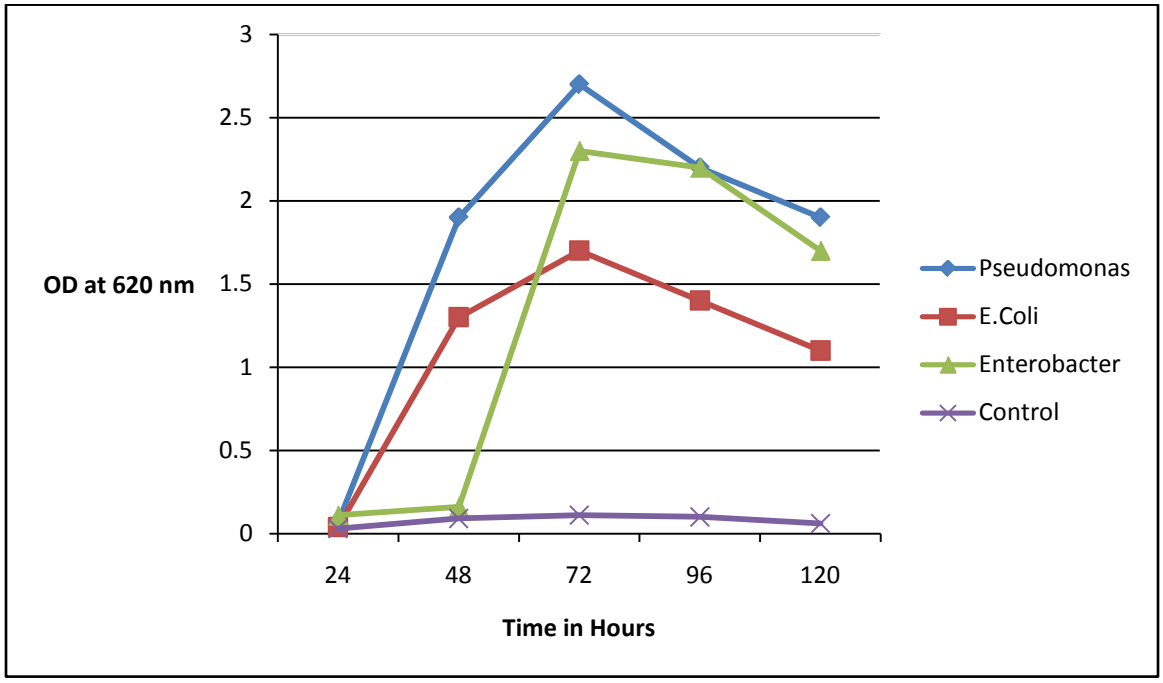

Fig. 1. Microbial Growth at 620nm

Table 3. Analysis of biochemical tests for isolated microorganisms

S. No.	Tests	*Pseudomonas* Sp.	*Enterobacter* Sp.	*E. Coli*
1.	Amylase	-ve	+ve	+ve
2.	Catalase	+ve	-ve	-ve
3.	Gram +ve/-ve	-ve	+ve	-ve
4.	Urease	-ve	-ve	+ve
5.	Indole	-ve	-ve	+ve
6.	MR	-ve	+ve	+ve
7.	VP		-ve	-ve
8.	Citrate	-ve	+ve	-ve

bioremediation. The pinnacle performance is achieved under a set of most favorable conditions. The factors affecting the success and rate of microbial biodegradation are nutrient availability, moisture content, soil reaction (pH), temperature, C/N ratio, soil texture etc. The present study was carried out to assess the capability of bacterial isolates to biodegrade oily sludge, a hazardous hydrocarbon waste generated by the petroleum industry. The bacterial isolates were found to adapt the local soil environment. However environmental factor play a vital role in the bioremediation of soil contaminated with oily sludge and the results obtained in this present investigation corroborate this view. All soil microorganisms require moisture for growth and functioning. Availability of water affects diffusion of water and soluble nutrients into and out of microbial cells. In the present investigation the moisture content recorded during the experiments ranged from 16.23% to 12.16%. A better degradation of PAH was recorded in soil samples PCS -1 having moisture content 12.16% as compared to PCS-2 with 16.23% moisture content. Excess moisture, in saturated soil, is undesirable because it reduces the amount of available oxygen for aerobic respiration. The soil water holding capacity between 45 and 85 percent is optimal for petroleum hydrocarbon degradation[9]. The water holding capacity of the contaminated soil samples under investigation in the present study was 57.3% in PCS-I and 60.3 in PCS-II, i.e. in the range suggested to be optimal for bioremediation[9]. Many Heavy metals also crutial for the rate of biodegradation as they act as a cofactor for enzymes activities. All the contaminated soil samples were found having heavy metals. The morphology and type of bacterial colonies were also investigated in cell counts experiments. One of the objectives of investigation to isolate and culture as many as microbial strains in suitable culture condition. For this reason, a first screening of strain was done after gram staining and microscopic examination for bacteria to eliminate apparently similar strains. As the results exhibited original sample were characterized with a very high diversity of microorganisms. The metabolic diversity of microorganisms in the natural environments is an important factor in the biodegradation of hydrocarbon. Extensive degradation of petroleum pollutants is generally accomplished by mixed microbial population, rather than single microbial species.

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