



Asian Journal of Research in Pharmaceutical Sciences and Biotechnology

Journal home page: www.ajrpsb.com



BIODEGRADATION OF PETROLEUM HYDROCARBON BY MICROBIAL POPULATION

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ABSTRACT

Petroleum hydrocarbon is naturally occurring energy source. It is found in lower surface of the earth and it is composed from rock minerals. The formation of petroleum is very long process with the degrading of plants and animals. Recently oil spilling is a major global problem that effect soil fertility and mineral deposition. The biodegradation is an alternative pathway to remove the petrochemical pollution. In this study the two bacterial strain were isolated i.e. *Pseudomonas putida* and *Bacillus cereus*. The crude oil degradation ability of bacterial strains was analyzed through clear zone formation. Among this study highest zone of exhibition was noted in *Pseudomonas putida* (6 mm in diameter) compared than *Bacillus cereus* (4 mm in diameter).

KEYWORDS

Hydrocarbon, *Pseudomonas putida* and *Bacillus cereus*.

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INTRODUCTION

Petroleum is naturally occurring energy source on earth. It contains various hydrocarbon and other chemicals. It consist complex mixture of hydrocarbon. It is extracted in the form of crude oil and mixed with various gases and trace minerals. After refinement process it forms various petrochemical products like gasoline, jet fuel, diesel fuel, kerosene etc. the soil contamination may result various health contamination and depleting the soil nourishment and fertility. It may develop global problem.

Biodegradation may refer to detoxification and removal of pollutants with the help of microorganism

due to their diverse metabolic activities. It is natural occurring cost effective process that cleans the environment. The natural population of microorganism has a tendency to follow various biochemical pathways to eliminate the pollutants from environment. The hydrocarbon degradation involves step by step therefore it provides energy that is utilized by microbial population.

MATERIAL AND METHOD

Sample Collection

The contaminated soil were taken from an automobile work shop in Bhilai, Chhattisgarh.

Media Preparation

Inorganic nitrogen and phosphorus salts were prepared and added separately to BM and ASW⁴ (Table No.1 and 2).

Isolation and Identification of Microbes from Soil Samples

The serial dilution was performed and inoculated the sample on medium. After inoculation they were incubated at 37°C for 24 hours. The bacterial colonies were obtained and performed biochemical characterization^{2,3} (Table No.3) through Biochemical confirmation test Bergey’s manual of systemic bacteriology classification.

Extraction of Residual Oil

The 50 ml volume of medium that contain 2% crude oil including acidified with HCl. The solvent hexane was used in the volume of 10ml and the flask was positioned on shaker at 120 rpm for 20 min. after 20 min. the solution was separated into two phase first, in aqueous phase and second phase is hexane phase. The aqueous phase was removed and the extraction was repeated with 10 ml of solvent. Temperature at 72°C
Analysis of Petroleum Hydrocarbon Biodegradation.

RESULTS AND DISCUSSION

The bacterial culture was obtained in various culture plate and performed biochemical testing by Bergey’s

Manual classification:

The crude oil degradation ability of bacterial strains was analyzed through clear zone formation. Among this study highest zone of exhibition was noted in *Pseudomonas putida* (6 mm in diameter) compared than *Bacillus cereus* (4 mm in diameter)¹⁰.

The biodegradation capacity of bacterial strain was more than high in petroleum contaminated soil as compare to other source. They were slowly degrading the soil polluted compound and become free from the hydrocarbon elements⁷.

In the present study the biodegradable bacterial strain utilizes the long chain of petroleum hydrocarbon as an energy sources. They degrade the bond between the carbon atoms and utilize the energy¹¹.

Generally they follow xenobiotic degradation pathway and cleavage pathway for the metabolic process and gain the energy by oxidation process. Sometimes they have limitations and any other factors which regulate the degradation pathway^{8,12,13}.

Soil samples of garden soil and local automobile workshop were inoculated in a jar containing 500ml minimal salts media (MSM). 5 ml of engine oil was used as hydrocarbon carbon source and left alone for 4 months under room conditions¹.

The tendency of the degradation of petrochemicals are comparatively high in bacteria and then in yeast and fungi. The reported efficiency of biodegradation ranged from 6%⁶ to 82%¹⁴ for soil fungi, 0.13%⁶ to 50%¹⁴ for soil bacteria, and 0.003%⁹ and to 100%⁵ for marine bacteria.

Table No.1: Basal Medium (BM)

S.No	Component	Concentration(g/l)
1	K ₂ HPO ₄	0.5g
2	NH ₄ Cl	2.0g
3	KNO ₃	2.0g
4	MgSO ₄	0.2g
5	Agar	15g

Table No.2: Artificial Seawater (ASW)

S.No	Component	Concentration(g/l)
1	NaCl	23.4g
2	KCl	0.75g
3	MgSO ₄	7g

Table No.3: Biochemical Characteristics

S.No	Biochemical Characteristics	Isolated Bacterial Colonies	
1	Colour of the colony	White	White
2	Shape of the cell	Bacillus	Rod
3	Gram s Staining	Positive	Negative
4	Motility	Motile	Motile
5	Indole	Negative	Negative
6	Methyl red	Negative	Negative
7	Voges-proskauer test	Negative	Negative
8	Citrate utilization test	Positive	Negative
9	Urease hydrolysis	Variable	Negative
10	Oxidase	Variable	Positive
11	Catalse	Positive	Negative
12	Carbohydrate fermentation test	A/G	A
13	Glucose	A/G	-
14	Dextrose	A/G	A
15	Maltose	Negative	Negative

CONCLUSION

Petroleum hydrocarbon is natural product which may be degrading by microbial population. A bacterial formulation consisting 2 strains isolated and purified from the selected soil sample. In this study the petroleum compound were degraded by *Pseudomonas putida* and *Bacillus cereus*. Generally they follow xenobiotic degradation pathway and cleavage pathway for the metabolic process and gain the energy by oxidation process.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Department of Life Science, Dr. C.V. Raman University, Bilaspur, Chhattisgarh, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Shweta Sao and Yogesh Deshmukh. Biodegradation of petroleum hydrocarbon by microbial population, *Asian Journal of Research in Pharmaceutical Sciences and Biotechnology*, 4(2), 2016, 53-56.