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Research Paper

Isolation and Identification of Naphthalene Degrading Microbe from Petroleum Contaminated Soil

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Abstract: *Naphthalene is a simplest Polycyclic Aromatic Hydrocarbon (PAH). PAHs are major contaminants of environment, associated with common anthropogenic activities such as oil refineries and incomplete combustion of fossil fuels. PAHs are toxic, mutagenic and carcinogenic. In the present study, bacterial strain was isolated from oil-spilled sediments were selected due to their capacity of growth in the presence of naphthalene as sole carbon and energy source. The isolates were identified by biochemical tests. Through this strain degradation of naphthalene were done. The strain belonged to Bacillus subtilis. Our results showed that the average degradation rates of naphthalene by Bacillus subtilis were recorded maximum in sample 3. According to ability of Bacillus subtilis in the degradation of naphthalene, it is recommended to use these species for remediation of oil compounds in contaminated sites.*

Keywords: Naphthalene, *Bacillus*, oil- spilled, degradation, remediation.

Introduction

Today, one of the major environmental problems is hydrocarbon contamination. This is the resulting from the activities related to petroleum refineries, accidental releases of petroleum products and some natural phenomenon are of meticulous alarm in the environment. The major source of energy for industries and daily life are petroleum based products [1]. Naphthalene, the simplest PAH, has long been used as a model compound in PAH bioremediation studies. Common naphthalene-degrading bacteria include *Pseudomonas spp., Vibrio spp*., *Mycobacterium* spp., *Marinobacter spp*, *Sphingomonas spp., Micrococcus spp.*[2]. Although many naphthalene-degrading bacteria have been isolated, these bacteria may thrive in one environment but may not be able to compete with other micro-organisms in another environment, as environmental conditions impose a selection pressure on specific types of bacteria[3].

Acute exposure of humans to naphthalene by inhalation, ingestion, and dermal contact is associated with hemolytic anemia, damage to the liver, and, in infants, neurological damage[4]. Cataracts have been reported in humans acutely exposed to naphthalene by inhalation and ingestion. Cataracts have also been reported in animals following acute oral exposure. Biodegradation of naphthalene can be achieved using adapted microbial consortium isolated from petrochemical contaminated environment. PAH bioremediation is considered an effective and environmentally benign cleanup technology as it involves the partial or complete bioconversion of these pollutants to microbial biomass, carbon dioxide and water[5].

Material and Methods

This research work was carried out in the period of September 2015.

Composition of media

Minimum Essential Media (MEM)
Constituents- Yeast extract, NaCl, Agar, Casein, Distilled water.

Soil sample collection

The soil samples were collected from 5 different petrol pumps in Indore. The ground was dug 4 cm above from the ground and soil was collected in sterile polythene bags.

Isolation of organism by agar plating method

MEM media, 5 petri plates and 5 test tubes with saline were sterilized. To the test tubes containing saline a pinch of different soil samples were mixed. To the sterile petri plate 10ml of media was poured and allowed to solidify. Then pour 0.1ml of soil sample and spread with spreader and then incubated at 37°C for 48 hours.

Pure culturing of the organism

The test tubes and MEM media were sterilized. The MEM media was poured into the tubes and allowed to solidify in slanting position. A loop full of culture from the culture plate were taken and streaked into the tubes. The tubes were incubated at 37°C for 24 hour.

Maintenance of pure cultures

Pure cultures were maintained by sub-culturing at intervals of every two days followed by incubation at 37°C for 24 hours. After which the cultures were preserved at 4°C.

Identification of organism

Gram staining was performed for the identification of bacteria.

Various other biochemical tests were performed such as indole production test, methyl red test, voges proskauer test, catalase test [6].

Evaluate degradation of Naphthalene from isolated organism

Prepare MEM broth for five samples and one control. Broth was cooled down. Add 10 mg naphthalene in all samples. Then add isolated pure culture in all samples containing naphthalene except one for control. Incubated in Shaker Incubator at 120-150 rpm, 37°C, for 24hrs., and degradation was estimated by spectrophotometer at 600 nm every 24 hours by taking supernatant through centrifugation under 10,000 rpm for 10 minute[3].

Results and Discussion

After 24hr of incubation whitish colony was observed on MEM agar plate. The pure cultures were maintained on MEM agar slant.

In this study bacterial species, *B. subtilis* strain were isolated to evaluate the ability of these bacteria for degradation experiment and their growth in the presence of naphthalene as sole source of carbon and energy. Bioremediation is the strategy that involves introducing indigenous Microorganisms to the contaminated site to detoxify and degrade environmental contaminants.

Table 1: Morphological and biochemical characteristic of bacterial strain

Characteristics	Bacterial strain
Gram Staining	Gram positive
Shape	Rod
Oxygen Requirement	Aerobic
Catalase	Positive
Indole Production	Positive
Methyl red test	Positive
Voges Proskauer Test	Negative

Table 2: Percentage of normal degradation of naphthalene by *Bacillus sp.*

Time	Sample 1 (in %)	Sample 2 (in %)	Sample 3 (in %)	Sample 4 (in %)	Sample 5 (in %)
24 Hours	3.81±0.004	2.86±0.001	4.83±0.008	3.01±0.001	2.65±0.003
48 Hours	5.71±0.016	1.91±0.002	6.62±0.004	6.60±0.001	5.91±0.001
72 Hours	9.52%±0.695	6.67±0.002	8.81±0.004	10.31±1.460	7.98±0.001

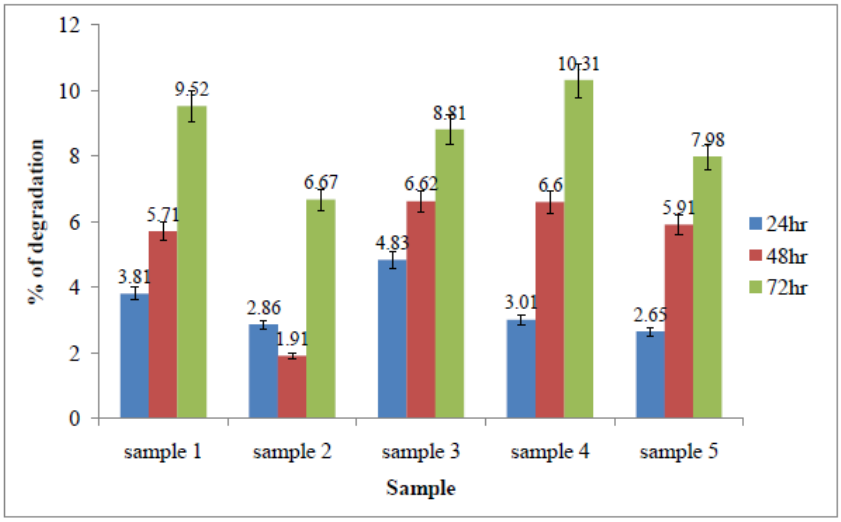

Figure 1: Percentage of normal degradation of naphthalene by *Bacillus sp.*

Several successful bioaugmentation cases have been documented[7].The ability of these bacteria to degrade oil hydrocarbons has been reported by several researchers, they isolated fifteen crude oil degrading *Bacillus sp.* The results demonstrated that *Bacillus* sp. can utilize crude oil as a carbon and energy source[8]. Furthermore, this study has demonstrated a very good biodegradation capability of crude oil hydrocarbons and industrial waste by bacterial consortium. The ability of isolated bacteria was determined by measuring the amount of degraded naphthalene. This species *B. subtilis* strain could degrade naphthalene. The effectiveness of bacterial isolates *B. subtilis* in the restoration of oil-field contaminated with PAHs was studied. Results showed that *Bacillus* degraded PAHs. A study indicating that *B. subtilis* was a dominant microorganism for degrading naphthalene.

Conclusion

Bacillus sp. was isolated and identified from the five different soil samples. The efficiency of the bacteria to degrade naphthalene was studied and it found to degrade PAH. In this present study it is proved that biodegradation of naphthalene from *Bacillus subtilis* collected from soil sample it was identified as gram as *Bacillus sp.* by gram staining and by biochemical test. We can suggest *Bacillus subtilis* as appropriate organisms used in areas polluted by naphthalene. Although, many strains have been previously isolated for naphthalene biodegradation, the current research work is suggested that this organism proved to be better than the previously isolated strains for the biodegradation of naphthalene.

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References

1. Jain P.K. and Bajpai V., Biotechnology of bioremediation-a review, *Int. J. Environ. Sci.*, **3(1)**, 535-549 **(2012)**

2. Rockne K. J., Chee-Sanford J. C., Sanford R. A., Hedlund B. P., Staley J. T. and Strand S. E., Anaerobic naphthalene degradation by microbial pure cultures under nitrate-reducing conditions, *Applied and Environmental Microbiology*, **66(4)**, 1595-1601 **(2000)**

3. Pawar A. N., Ugale S. S., More M. G., Kokani N. F. and Khandelwal S. R., Biological degradation of naphthalene: A new era, *Journal of Bioremediation & Biodegradation.*, **(2013)**

4. Amoore J. E. and Hautala E., Odor as an ald to chemical safety: odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. *Journal of applied toxicology.*, **3(6)**, 272-290 **(1983)**

5. Ahn Y., Sanseverino J. and Sayler G. S., Analyses of polycyclic aromatic hydrocarbon-degrading bacteria isolated from contaminated soils, *Biodegradation*, **10(2)**, 149-157 **(1999)**

6. Rao P. R. and Kavya P., Production, Isolation and Purification of Peroxidase Using Bacillus Subtilis, *IPCBEE*., **64(5)**, 21-27 **(2014)**

7. Grund E., Denecke B. and Eichenlaub R., Naphthalene degradation via salicylate and gentisate by Rhodococcus sp. strain B4, *Applied and Environmental Microbiology*, **58(6)**, 1874-1877 **(1992)**

8. Sepahi A.A., Golpasha I.D., Emami M. and Nakhoda A.M., Isolation and characterization of crude oil degrading bacillus spp., *Journal of Environmental Health Science & Engineering*, **5(3)**, 149-154 **(2008)**.