BIOREMEDIATION OF CARCINOGENIC POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) RICH EFFLUENT FROM PETROCHEMICAL INDUSTRIES

Project Reference No.: *45S_BE_4546*

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Keywords:

Bioremediation, Fungal bioremediation, PAH, GCMS

Introduction:

Polycyclic Aromatic Hydrocarbons (PAHs) are potent environment pollutants which are ubiquitously distributed, having fused aromatic rings. PAHs are formed due to incomplete combustion of wood, coal, oil and gasoline. Presence of PAHs has been reported in crude oil, coal tar and asphalt. Main cause of soil and water pollution is the release of hydrocarbons accidentally or through human activities. PAHs are toxic, carcinogenic and mutagenic so their presence in environment is of great concern and has deleterious effect on human health. 16 PAHs have been listed as priority pollutants by United States Environment Protection agency (US EPA) and are monitored continuously in industrial effluents. PAHs are suspected carcinogens. They become genotoxic when activated by mammalian enzymes to reactive epoxides and quinones by enzymes such as cyctochrome P450 monoxygenase.

The fate of PAHs in soil and water is as follows; Volatilization, Absorption, leaching, Erosion relocates PAHs without altering structure. Biodegradation and oxidation reduction reactions alter the structure. Some process relocates PAHs into long-term storage without altering structure (absorption, diffusion into soil micro pores).

Bioremediation remains ecofriendly and effective way to remediate PAHs. Various species of bacteria, algae and fungi have been studied for their degradation ability. Bacteria are the most widely studied microorganisms in bioremediation. Fungal strains are less explored and in this study a fungal isolate is used for bioremediation of PAHs.

Objectives:

The proposed objectives to study bioremediation of carcinogenic PAHs are as follows:

(i) Screening of microorganisms to check degradation ability
(ii) Sub-culture and preparation of standardized spore suspension as inoculum

(iii) Design of degradation studies
(iv) Evaluation of degradation using UV- spectrometry
(v) Gas chromatography and mass spectroscopy (GCMS) analysis of extracted samples to analyze small metabolites generated during bioremediation
(vi) Elucidation of degradation pathway of PAH by microorganisms from GCMS results.

Methodology:

All PAHs used in the studies were purchased from Tokyo Chemical Institute (TCI), n-hexane used for liquid-liquid extraction, media compositions were prepared from chemicals available in the inventory. Orbital shaker (ORIENS scientific innovations Pvt. Ltd,) was used for shake flask studies. Thermoscientific (Evolution 201) UV-visible spectrometer was used for absorbance studies.

The isolate named NR-2 was isolated from soil samples from Western Ghats (Karnataka, India) and the organism was grown on mineral salt media with PAH as carbon source. The isolate was incubated for 7-10 days and as subcultured in potato dextrose broth. Once the organism showed luxuriant growth and started sporulation, a spore suspension was prepared (2-5% saline and tween-20). Spore count was taken by hemocytometer method. The obtained spore count was standardized to 105 spore/ml and this was used as inoculum.

Shake flask study was designed and analyses was carried out on 3, 6, 9, 12 and 15th day after inoculation. A control with PAH and without inoculum and a control with inoculum and without PAH was also used. All the experiment was carried out in triplicates. After every 72 hour media was extracted by multi-stage liquid-liquid extraction using n-hexane as an extraction solvent. Later the nonaqueous was used for UV analysis.

Absorbance was measured for each tubes using UV spectroscopy and decrease in absorbance was correlated for bioremediation. The extracted samples were stored in screwcap test tubes and were sent for GCMS study. From GCMS results, pathway of degradation was derived.

Results and Conclusions:

When media was supplemented with PAHs as carbon source, luxuriant growth was seen with anthracene as an exception with no growth. Luxuriant growth when PAHs was supplemented suggests that the microorganism was effectively utilizing PAHs as a source of carbon.

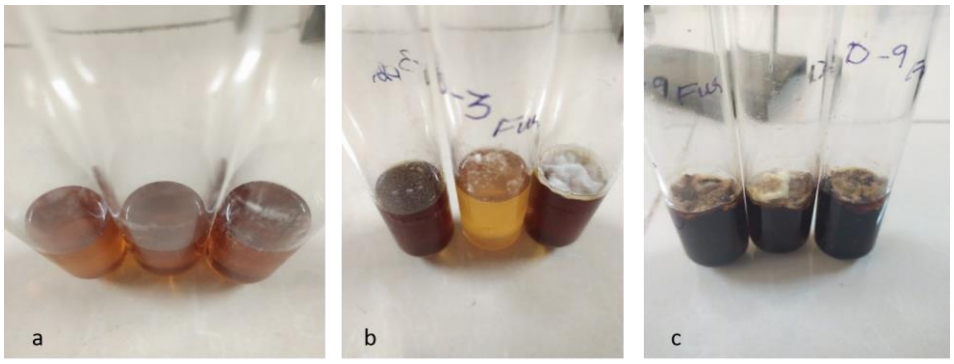
Table 1: Growth of NR-2 in media supplemented with different PAH

Name of the PAH	Growth of the organism
Anthracene	-
Fluorene	+++
Acenaphthene	+++
Fluoranthene	+++

UV spectrogram showed changes in peaks obtained suggesting that the microorganism is utilizing the PAHs and certain structural changes might have occurred. To analyze the structural changes the extracted PAHs will be sent to GCMS.

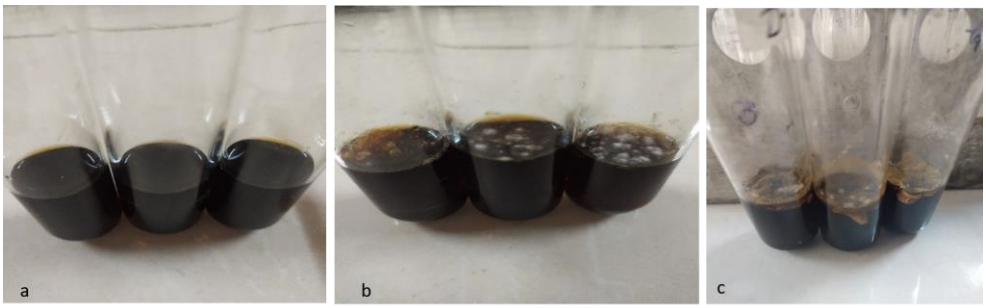
Images of the test tubes showing the growth of the fungi in successive days:

1. Fluorene



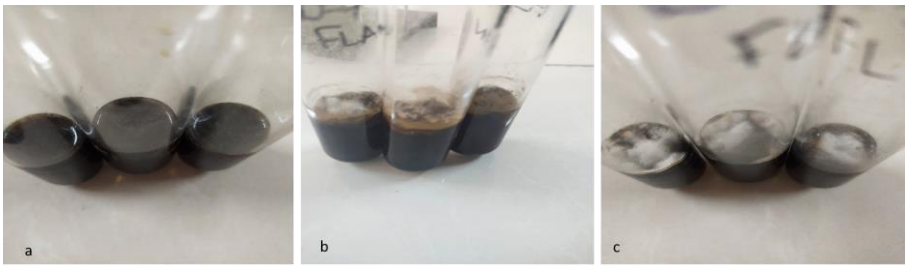
a) Control with Fluorene b) Day 3 degradation c) Day 9 degradation

2. Acenaphthene



a) Control b) Day 3 degradation c) Day 9 degradation

3. Fluoranthene



a) Control with Fluoranthene b) Day 3 degradation c) Day 9 degradation

Scope For Future Work:

The fate of PAH in nature is of great environmental concern due to its mutagenic and carcinogenic toxicity. Biodegradation of PAHs and other xenobiotic compounds depend upon microorganisms to either transform or mineralize them to CO2 and H2O. The present study notes that the effective depolluting of PAH from contaminated site with the help of bioremediation appear to be most efficient and cost effective environmental friendly to decontaminate PAH.

Fungal strains are less explored in bio0remediation of PAH but fungal species have higher remediation efficiency as they produce wide range of extracellular enzymes. PAHs contaminate almost all the niches as they can be carried away by wind or water. As PAH are carcinogenic and can contaminate any niche decontaminating them in there source is necessary. This project provides scope for efficient biodegradation of PAHs in an ecofriendly way.