Journal of Environmental Management 181 (2016) 728–736

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Enhanced biodegradation of PAHs by microbial consortium with different amendment and their fate in *in-situ* condition

Anamika Sharma [a], Shashi Bala Singh [b], Richa Sharma [a], Priyanka Chaudhary [a], Alok Kumar Pandey [a], Raunaq Ansari [b], Venugopal Vasudevan [a], Anju Arora [a], Surender Singh [a], Supradip Saha [b], Lata Nain [a, *]

[a] Division of Microbiology, Indian Agricultural Research Institute, New Delhi 110012, India
[b] Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi 110012, India

ARTICLE INFO

Article history:
Received 23 January 2016
Received in revised form
8 August 2016
Accepted 9 August 2016
Available online 21 August 2016

Keywords:
Bioremediation
Streptomyces
Consortium
Phanerochaete
Bioaugmentation

ABSTRACT

Microbial degradation is a useful tool to prevent chemical pollution in soil. In the present study, *in-situ* bioremediation of polyaromatic hydrocarbons (PAHs) by microbial consortium consisting of *Serratia marcescens* L-11, *Streptomyces rochei* PAH-13 and *Phanerochaete chrysosporium* VV-18 has been reported. In preliminary studies, the consortium degraded nearly 60–70% of PAHs in broth within 7 days under controlled conditions. The same consortium was evaluated for its competence under natural conditions by amending the soil with ammonium sulphate, paddy straw and compost. Highest microbial activity in terms of dehydrogenase, FDA hydrolase and aryl esterase was recorded on the 5th day. The degradation rate of PAHs significantly increased up to 56–98% within 7 days under *in-situ* however almost complete dissipation (83.50–100%) was observed on the 30th day. Among all the co-substrates evaluated, faster degradation of PAHs was observed in compost amended soil wherein fluorene, anthracene, phenanthrene and pyrene degraded with half-life of 1.71, 4.70, 2.04 and 6.14 days respectively. Different degradation products formed were also identified by GC-MS. Besides traces of parent PAHs eleven non-polar and five polar products were identified by direct and silylation reaction respectively. Various products formed indicated that consortium was capable to degrade PAHs by oxidation to mineralization.
© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Polyaromatic hydrocarbons (PAHs) are highly toxic and hazardous xenobiotic compounds, belonging to a class of organic compounds consisting of carbon and hydrogen which are made of two or more fused aromatic rings in different structural forms. They have been classified as most recalcitrant as they may persist for long time in soil because of their low water solubility and hydrophobicity. The United States Environmental Protection Agency (USEPA) has proposed 16 priority pollutants with pyrene, naphthalene, anthracene, fluorene and phenanthrene as the most abundant and hazardous pollutants for mankind. PAHs enter in environment by natural and anthropogenic sources during thermal geologic reactions associated with fossil-fuel and mineral production, burning of vegetation in forests, bush fires, fuel combustion, automobiles, spillage of petroleum products, and plastic waste incineration (Antizar-Ladislao et al., 2006). The high molecular weight PAHs are mutagenic and carcinogenic in nature and cause various health problems, specially gastrointestinal cancer when they enter into the human body through consumption of contaminated food, drinking water, inhalation of cigarette smoke, automobile exhausts, and contaminated air from occupational settings (Diggs et al., 2011).

Due to the poor solubility in water, the level of PAH compounds continuously keeps on increasing in soil. Bioremediation or microbial degradation approach is being used since long time to degrade PAHs. Various bacterial genera like *Pseudomonas*, *Sphingomonas*, *Mycobacterium*, *Sphingobium* etc. have been reported for the degradation of PAHs by different mechanisms including biosurfactant production and biofilm formation around the PAH compounds (Johnsen and Karlson, 2004). Microorganisms degrade the PAHs enzymatically and convert them to less toxic products. To ensure the environmental safety, it is also important to identify

* Corresponding author. Principal Scientist, Division of Microbiology, Indian Agricultural Research Institute, New Delhi 110012, India.
E-mail address: latarajat@yahoo.co.in (L. Nain).

http://dx.doi.org/10.1016/j.jenvman.2016.08.024
0301-4797/© 2016 Elsevier Ltd. All rights reserved.

these metabolites of PAHs after biodegradation. In general, all aerobic microorganisms catabolize PAHs by the action of dioxygenase/monooxygenase enzyme, through oxidation of aromatic ring or by addition of molecular oxygen in aromatic nucleus. These intermediates further convert to cis-dihydrodiols resulting into catechols. Finally catechols are converted to aliphatic acids or intermediates of TCA cycle (Chauhan et al., 2008). For complete and effective degradation of PAH mixture having high and low molecular weight PAHs, different microbial partners are more suitable because bacteria alone may not be able to degrade all these PAHs completely. Bacteria is efficient in utilizing and degrading lower molecular weight PAHs but high molecular weight PAHs are thermodynamically stable and hydrophobic in nature which decreases its degradability by bacteria (Bezalel et al., 1996). Various ligninolytic fungi like *Phanerochaete chrysosporium, Pleurotus ostreatus* etc. have shown better dissipation of high molecular weight PAHs cometabollically with bacteria (Aranda, 2016; Wang et al., 2012).

In earlier reports from our laboratory, Pandey et al. (2012) reported high PAH degradation potential of *Serratia marcescens* L- 11. This strain has the ability to produce catechol 1,2-dioxygenase, lipase and biosurfactant which are responsible for enhanced PAH degradation by providing better solubility. Similarly, a biosurfactant producing actinomycetes *Streptomyces rochei* PAH-13 was able to degrade 28–92% PAHs within 15 days of incubation (Chaudhary et al., 2011). A white rot fungus, *Phanerochaete chrysosporium* VV-18 isolated from oil contaminated soil was found to be capable of producing ligninolytic enzyme as well as found to be an efficient PAHs degrader (unpublished results). In soil, there is always a mixture of PAHs, and application of single microbe may not be effective. For this consortium of various microbes may be better suited for bioremediation. Therefore, aim of present study was to develop a potent microbial consortium of these selected microorganisms to achieve enhanced degradation of PAHs. To the best of our knowledge, no published information is available on the tripartite association of fungi-bacteria-actinomycetes in PAH degradation in soil.

For effective bioremediation, environmental conditions should also be conducive for the growth and activity of introduced/inoculated microbes. Biostimulation through the addition of nutrients also create a favorable environment for the growth of microorganisms resulting into effective degradation of the PAH mixture (Bach et al., 2005; Andreolli et al., 2015). This strategy stimulates the microbial activity of indigenous and introduced microflora to enhance the bioremediation process. Therefore, the effect of different co-substrates eg. paddy straw, compost and ammonium sulphate were also evaluated for *in-situ* degradation of PAHs. The degradation products of PAHs were also characterized to decipher the metabolic mechanism of degradation.

2. Material and methods

2.1. Substrates

For the experiment, paddy straw and loamy top soil was collected from the farms of Indian Agricultural Research Institute (IARI), New Delhi, India. Compost was obtained from the composting pits of Division of Microbiology, IARI, which was prepared by paddy straw as substrate. Physiochemical analysis of soil, compost and paddy straw has been given in Supplementary Table 1.

2.2. Microorganisms-compatibility testing and consortium development

A bacterium, *S. marcescens* L-11 (NCBI accession no. EF591778), actinomycetes, *S. rochei* PAH-13 (NCBI accession no. GQ904711) and white rot fungus, *P. chrysosporium* VV-18 (NCBI accession no. JX481075) isolated previously from different contaminated sites were used in the present study. The bacterium and actinomycete were maintained on nutrient agar, while the fungus was maintained on Potato Dextrose Agar (PDA).

The compatibility test of the three cultures was performed by streaking them diagonally on nutrient agar medium. The plates were incubated at 30 ± 1 °C for 48 h and observations for growth and compatibility were recorded.

2.3. Microbial degradation of PAHs in broth

The selected cultures were tested for PAH degradation in Bushnell and Haas (B&H) minimal medium (Bushnell and Haas, 1941) fortified with PAHs mixture as sole C source under submerged conditions, pH of medium was adjusted to 6.8 ± 0.3. PAHs were dissolved in acetone to make a stock of 1000 μg mL^{-1} solution and PAHs @ 50 μg mL^{-1} each (fluorene, anthracene, phenanthrene and pyrene) were added from the stock solutions in a conical flask and solvent evaporated under laminar flow. 25 mL sterilized B&H medium was added to flask resulting in 200 μg mL^{-1} total PAH concentration in each flask. Different treatments were tested with following microbial combinations in triplicate:

A: *S. marcescens*, **B:** *S. rochei*, **C:** *P. chrysosporium*, **D:** *S. marcescens* + *S. rochei*, **E:** *S. marcescens* + *P. chrysosporium*, **F:** *S. rochei* + *P. chrysosporium*, **G:** *S. marcescens* + *S. rochei* + *P. chrysosporium*.

The medium was inoculated with 1% v/v inoculum of respective cultures. The inoculum of *S. marcescens* L-11, *S. rochei* PAH-13 contained 10^{11} cfu mL^{-1} while *P. chrysosporium* consisted of 10^{7} cfu mL^{-1}. All the treatments were taken in triplicate. Flasks were incubated at 30 ± 1 °C for seven days in shaking incubator (150 rpm) for proper aeration. Samples were withdrawn on 3rd, 5th and 7th day after incubation for estimation of microbial activity by fluorescein diacetate hydrolase (FDA hydrolase) assay (Swisher and Carroll, 1980), cell density at wavelength of 600 nm, and soluble protein content (Lowry et al., 1951).

2.4. Quantification of residual PAHs by HPLC

Amount of PAHs was quantified by HPLC on the 7th day. PAHs were extracted from media with dichloromethane (DCM; 50+30+20 mL) after making it saline (15% aqueous NaCl 50 mL). The DCM extract was passed through anhydrous sodium sulphate (15 g) and solvent concentrated on rotary vacuum evaporator. The residual solvent was exchanged with acetonitrile under nitrogen stream. The solution was filtered through 0.22 μm membrane filter before injecting into HPLC. HPLC analysis was carried out using Hewlett Packard HPLC instrument (series 1100) equipped with degasser, quaternary pump and photo diode-array detector connected with rheodyne injection system (20 μL loop). The stationary phase consisted of RP-18 (PAH) packed stainless steel column (Merck). Chromatogram was recorded in a Windows' NT based HP Chemstation programme. Acetonitrile: water (60:40) was used as mobile phase at a flow rate of 0.5 mL min^{-1}. HPLC analysis was performed at wavelength of 246 nm, which was detected for absorption maxima of all four compounds using isoplot option provided in the system. Each run was repeated thrice and response was measured in terms of peak areas. All the estimations were performed in triplicate and mean was calculated as per standard procedures. Error values at 5% level are depicted in the graphs as bars.

Download English Version:

https://daneshyari.com/en/article/7479848

Download Persian Version:

https://daneshyari.com/article/7479848

Daneshyari.com