

# Biodegradation of Polycyclic Aromatic Hydrocarbons using Bacterial Consortium

Gauri Gupta<sup>1</sup> and Dr. Vipin Kumar<sup>2</sup> and A. K. Pal<sup>3</sup>

<sup>1</sup>Junior Research Fellow Department of Environmental Science and Engineering Indian School of Mines, Dhanbad-826004, Jharkhand

<sup>2,3</sup>Department of Environmental Science and Engineering Indian School of Mines, Dhanbad-826004, Jharkhand  
E-mail: <sup>1</sup>[gauri.gupta27@gmail.com](mailto:gauri.gupta27@gmail.com), <sup>2</sup>[vipinmicro1@gmail.com](mailto:vipinmicro1@gmail.com), <sup>3</sup>[palasim2003@yahoo.co.in](mailto:palasim2003@yahoo.co.in)

**Abstract:** The waste from oil industry is a major threat to the environment as it contains various toxic, mutagenic and carcinogenic substances like Polycyclic Aromatic Hydrocarbons (PAHs). PAHs are a class of diverse organic compounds with two or more fused benzene rings in a linear, angular or cluster arrangement. Removal of PAHs is of concern as these are persistent pollutants with ubiquitous occurrence and detrimental biological effects. Available physical and chemical methods are neither eco-friendly nor cost-effective therefore, biological methods are best suited for biodegradation of PAHs. Such methods requires less chemicals, less time and less input of energy and are also cost-effective and eco-friendly. The toxic PAH compounds can be converted into non-toxic and simpler ones using naturally occurring microbes like algae, bacteria, fungi in a process called biodegradation. Various researches have been found in the literature regarding PAHs degradation using pure bacterial cultures. But, recently the use of bacterial consortium has found to be more efficient than pure bacterial cultures. Bacterial consortium is an assemblage of several bacterial species appropriate for degradation. The synergistic interactions amongst its species leads to maximum possible degradation leading to higher degradation rates. This review focuses on the enhancement in biodegradation of hazardous PAHs using bacterial consortium.

**Keywords:** Polycyclic aromatic hydrocarbons; Bacterial consortium; Biodegradation; PAHs degrading microorganisms.

## 1. INTRODUCTION

The pollution due to petroleum hydrocarbon is a pervasive, global problem. Crude oil or petroleum is a complex mixture of aliphatic and aromatic hydrocarbons. Polycyclic aromatic hydrocarbons (PAHs) are a class of diverse organic compounds with two or more fused benzene rings in a linear, angular or cluster arrangement [1]. PAHs are included in the US Environmental Protection Agency (EPA) and European Community (EC) priority pollution list. EPA currently regulates 16 PAH compounds as priority pollutants in water and as “total PAHs” in contaminated soil and sediments [7]. Physico-chemical properties and molecular weight of PAHs varies with the number of rings in the molecule. Increase in molecular weight of PAHs leads to decrease in Chemical reactivity, aqueous solubility and volatility of PAHs. PAHs are

ubiquitous in nature and are found throughout the environment in air, water and soil. PAHs are divided into low and high molecular weight PAHs. Low molecular weight PAHs are less hydrophobic and therefore more soluble than high molecular weight PAHs. This is why high molecular weight PAHs remain bound to soil organics making themselves less available for natural degradation [6]. In soil, PAHs remains absorbed by soil organic matter. Soil serves as a source for low molecular weight PAHs and as a sink for higher molecular weight PAHs [3] The persistence, toxicity and carcinogenicity of PAH molecules draws public concern to decontaminate PAH polluted sites [13].

## 2. SOURCES OF PAHS IN ENVIRONMENT

Natural sources of PAHs include their formation as, exudates from trees forests and rangeland fires, fungi and bacteria. In nature PAHs remains predominantly distributed as components of plant oils, cuticles of insects, components of surface waxes of leaves and lipids of microorganisms. PAHs are formed naturally during thermal geologic reactions associated with fossil-fuel and mineral production. Anthropogenic sources like fuel combustion, automobiles, spillage of petroleum products, electric fuel generation, internal combustion engines and waste incinerators are significant sources of PAHs into the environment [5]. Anthropogenic sources of PAHs are the major cause of environmental pollution and hence the focus of many bioremediation programmes. PAHs remains deposited in the atmosphere through widely dispersed sources covering significantly the land surface area. At such sources PAHs is found to be absorbed strongly to soil particles.

## 3. BIODEGRADATION OF PAHS

The environmental fate of PAHs in both terrestrial and aquatic ecosystem remains affected by microbial degradation. Biodegradation is a viable method for degradation of organic pollutants. Some of the soil bacteria consume petroleum

hydrocarbons as a food source and degrade them into harmless substances like CO<sub>2</sub>, H<sub>2</sub>O, fatty acids. The biodegradation of PAHs in soil involves two steps. First step involves the uptake of PAHs by soil microbes which remains affected by several factors like bioavailability of PAHs in soil (aqueous phase), properties of soil and environmental conditions. The second step involves the microbial PAHs degradation, which mostly depends on the biological ability of microbes [19, 20, 21]. The desorption of PAHs from soil organics determines its degradation rate [34]. Several additional factors affecting rate of degradation includes pH, temperature, presence of oxygen, nutrient availability. The biodegradation of PAHs is cost-effective and eco-friendly (as it prevents environmental damage during transportation of contaminants). Structure of PAHs in crude oil is very complex and pure cultures of bacteria do not have the ability to degrade different classes of PAHs. Therefore, a mixture of various microbes (consortium) is applied. It has been found that the use of microbial consortium is more effective than pure cultures in degradation process [16].

### 3.1 Degradation of PAHs by bacteria

Bacterial degradation of PAHs involves the initial oxidation step which includes the incorporation of both oxygen atoms into the aromatic ring to form *cis*-dihydrodiol and the reaction remains catalyzed by dioxygenase enzyme. These *cis*-dihydrodiols are dehydrogenated in the presence of enzyme dehydrogenases to form dihydroxylated intermediates. These intermediates are further metabolized via catechols to carbon dioxide and water. Bacterial species like *Pseudomonas*, *Rhodococcus*, *Alcaligenes*, *Sphingomonas* and *Mycobacterium* are capable of degrading PAHs.

Most of these bacteria have ability to grow on low molecular PAHs. But only in recent past, several bacteria have been isolated that are able to grow on four ring PAHs. Several literature has been found citing repeated isolation of *Mycobacteria* which is capable of degrading PAHs containing four or more fused aromatic rings. This is probably due to the hydrophobic cell surface of *mycobacterium* allows its adhesion to hydrophobic PAHs, thus facilitating mass transfer of the substrates inside the cells [4]. "Fig. 1. three main pathways for polycyclic aromatic hydrocarbon degradation by bacteria, fungi and algae (adapted from Mueller [11])".

## 4. MICROBIAL CONSORTIUM

Use of microbial consortium is becoming more prevalent than pure bacterial cultures as no single specie has the ability to metabolize more than two or three types of hydrocarbons (low degradation rates) whereas the synergistic interactions among the members of consortium may lead to complete degradation of the product increasing the degradation and mineralization rates. In a consortium it is possible that one species removes the toxic metabolites (that otherwise may hinder microbial

activities) of the species preceding it. It is also possible that the second species are able to degrade compounds that the first are only able to degrade partially. Rahman et al.<sup>14</sup> reported that the degradation rate of Bombay High crude oil by a mixed bacterial consortium was more than the pure bacterial cultures. Mixed consortium degraded 78% in case of Bombay High crude oil whereas pure cultures degraded lesser amounts (66% by *Pseudomonas* sp. DS10-129, 59% by *Bacillus* sp. DS6-86, 49% by *Micrococcus* sp. GS2-22, 43% by *Corynebacterium* sp. GS5-66, 41% by *Flavobacterium* sp. DS5-73). Muthuswamy et al.<sup>12</sup> studied the biodegradation of crude oil using bacterial consortium made of four strains. The mixed Consortium made of various strains of bacteria are found to be effective in degradation of bacterial consortium showed a degradation rate of 77% higher than the individual strains (69% by *Pseudomonas* sp. BPS1-8, 64% by *Bacillus* sp. IOS1-7, 45% by *Pseudomonas* sp. HPS2-5 and 41% by *Corynebacterium* sp. BPS2-6.

"Table 1. Comparative study of degradation of hydrocarbons by pure bacterial culture and mixed bacterial consortium".

Microorganisms produce several biosurfactants during the process of PAHs degradation. These biosurfactants help perform the solubilization of hydrocarbons in water, thus making it available for biodegradation. Biosurfactants have wide range of properties like reduction in surface tension, interfacial tension and critical micelle formation (CMC). These properties result in dissolution of hydrocarbons that occurs through micelle formation in micro-emulsions produced by biosurfactants.

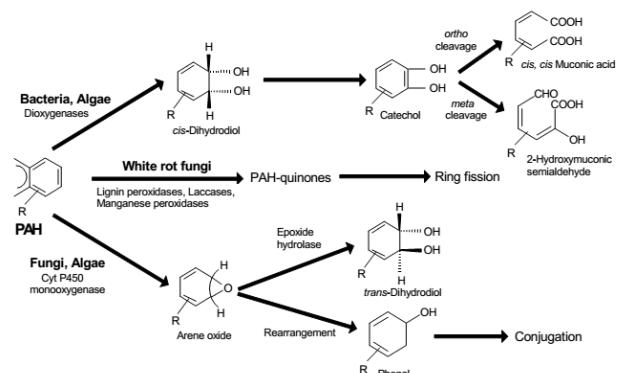
## 5. CONCLUSION

The current status of work done on biodegradation of ecologically toxic PAHs using different bacterial consortium has been critically reviewed. The environmental toxicity and persistence of PAHs has resulted in several laboratory based experiments to transform these substances into less hazardous/non hazardous substances with the use of microorganisms in the process called as biodegradation. Degradation of PAHs remains affected by several factors which needs to be addressed and explored. Several bacterial species isolated from contaminated sites are capable of degrading PAHs into simpler and less toxic substances. These species when combined (consortium) have performed better increasing the degradation rates. Though, there are several limitations that require further research like degradation of high molecular weight. Conditions favorable for anaerobic degradation of PAHs are yet to be explored and applied to clean several sub-surface PAHs contaminated sites.

## REFERENCES

- [1] Boonchan, S., Britz, M. L., and Stanley, G. A., "Degradation and mineralization of high-molecular-weight polycyclic aromatic hydrocarbons by defined fungal-bacterial cocultures", *Appl. Environ. Microbiol.*, 66, 2000,1007-1019.
- [2] Cerqueira, V. S., Hollenbach, E. B., Maboni, F., Vainstein, M. H., Camargo, F. A. O., Peralba, M. C. R., and Bento F. M., "Biodegradation potential of oily sludge by pure and mixed bacterial cultures", *Bioresour. Technol.* 102, 2011, 11003-11010.
- [3] Cousins, I. T., and Jones K. C., "Air-soil exchange of semi-volatile organic compounds (SOCs) in the UK", *Environ. Pollut.*, 102, 1998, 105-118.
- [4] Harayama, S., "Polycyclic aromatic hydrocarbon bioremediation design. *Curr Opin Biotechnol* 8", 1997, 268-273.
- [5] Haritash, A. K. and Kaushik C. P., Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): A review, *J. Hazard. Mater.*, 169, 2009, 1-15.
- [6] Harvey, R. G., "Polycyclic aromatic hydrocarbons", Wiley-VCH New York, N.Y. 1997.
- [7] Liu, K., Han, W., Pan, W. and Riley, J. T., "Polycyclic aromatic hydrocarbon (PAH) emissions from a coal-fired pilot FBC system", *J. Hazard. Mater.*, 84, 2001,175- 188.
- [8] MacLeod, C. J., Morriss, A. W., and Semple K. T., "The role of microorganisms in ecological risk assessment of hydrophobic organic contaminants in soils", *Adv. Appl. Microbiol.*, 48, 2001, 171-212.
- [9] Maeir, R. M., Pepper, I. L., and Gerba, P. C., "A textbook of environmental microbiology", Academic press San Diego, CA, 2000.
- [10] Manilal, V. B., and Alexander, M., "Factors affecting the microbial degradation of phenanthrene in soil", *Appl. Microbiol. Biotechnol.*, 35,1991. 401-405.
- [11] Mueller, J.G., Cerniglia, C.E., "Pritchard, P.H. "Bioremediation of environments contaminated by polycyclic aromatic hydrocarbons. In": Crawford, R.L., Crawford, D.L., (eds) Bioremediation: Principles and Applications, Cambridge University Press, U.K., 1996, pp 1215-1294.
- [12] Muthuswamy, S., Binupriya, A. R., Baik, S., and Yun, S., "Biodegradation of Crude Oil by Individual Bacterial Strains and a Mixed Bacterial Consortium Isolated from Hydrocarbon Contaminated Areas", *CLEAN - Soil Air Water.*, 36, 2008, 92-96.
- [13] Patnaik, P., "Hydrocarbon, aromatic. In: A Comprehensive Guide to the Hazardous Properties of Chemical Substances. New York: Van Nostrand Reinhold", 1992, 425-445.
- [14] Rahman, K. S. M., Thahira-Rahman, J., Lakshmanaperumalsamy, P., and Banat, I. M., "Towards efficient crude oil degradation by a mixed bacterial consortium", *Bioresour. Technol.*, 85, 2002, 257-261.
- [15] Shuttleworth, K. L., and Cerniglia, C. E., "Environmental aspects of polycyclic aromatic hydrocarbon biodegradation", *Appl. Biochem. Biotechnol.*, 54, 1995, 291-302.

- [16] Wu, M., Chen, L., Tian, Y., Ding, Y. and Dick, W. A., "Degradation of polycyclic aromatic hydrocarbons by microbial consortia enriched from three soils using two different culture media", *Environ. Pollut.*, 178, 2013,152-158.



**Fig. 1: The three main pathways for polycyclic aromatic hydrocarbon degradation by bacteria, fungi and algae (adapted from Mueller [12]).**

**Table 1: Comparative study of degradation of hydrocarbons by pure bacterial culture and mixed bacterial consortium**

Source	Bacterial species	Degradation rate (%)		References
		Bacterial species	Bacterial consortium	
Crude oil	Pseudomonas sp. BPS1-8	69%	77%	[12].
	Bacillus sp. IOS1-7	64%		
	Pseudomonas sp. HPS2-5	45%		
	Corynebacterium sp. BPS2-6	41%		
Oily sludge	Stenotrophomonas acidaminiphila	33.2%	51.8%	[2].
	Bacillus cibi	64.3%		
	Bacillus megaterium	39.6%		
	Pseudomonas aeruginosa	40.3%		
	Bacillus cereus	51.8%		
Crude oil	Pseudomonas sp. DS10-129	66%	78%	[14]
	Bacillus sp. DS6-86	59%		
	Micrococcus sp. GS2-22	49%		
	Corynebacterium sp. GS5-66	43%		
	Flavobacterium sp. DS5-73	41%		