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Nano-Enhanced Microbial Remediation of PAHs Contaminated Soil

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ABSTRACT: The emergence of polycyclic aromatic hydrocarbons (PAHs) from a variety of natural and anthropogenic sources, such as coal gasification and liquefaction plants, coke and aluminum production, catalytic cracking towers, and motor vehicle exhaust, among others, results in significant soil pollution, and a threat to human health, igniting a surge of interest in advanced research. Even though the cleanup of PAHs-contaminated areas received a great consideration. In the last decade, nanotechnology has exploded in popularity as a result of several unique properties of nanomaterials, and remediation is no exception. Thus, nano-enhanced bioremediation reported to act as a viable and effective strategy for PAHs remediation. Further, the integration of nano-enabled materials with microorganisms emerged as a promising biodegradation approach for PAHs remediation. As a result, the focus of this mini review is on depicting the possible roles of various nanomaterials in decontaminating PAHs as a green strategy by boosting the efficacy of microbial functionality, and mechanism of nanoparticles-microbes interaction in PAHs degradation. The future perspective of nano-enhanced microbial remediation of PAHs in realistic environments are also discussed.

KEYWORDS: Nanoparticles (NPs), polycyclic aromatic hydrocarbons (PAHs), biodegradation, phytoremediation, mechanism

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Introduction

With the emergence of industrialization and progressive technologies, there is a continuous enhancement of persistent xenobiotic contaminants in the environment which threaten human beings, the ecosystem, and other organisms. Among xenobiotics, hydrophobic polycyclic aromatic hydrocarbons (PAHs) are the most persistent, mutagenic, carcinogenic, and ubiquitous pollutants. The mutagenic property of PAHs explain that the compound is metabolically active and has ability to cause mutation when they interact with living organisms. The next important property of PAHs is carcinogenic which cause cancer in various organs of human being due to the parent compounds or metabolites and if the compound cause cancer, then it should be mutagen (Pashin & Bakhitova, 1979). There are 16 PAHs that are considered a priority pollutant by US Environmental Protection Agency (EPA) (S. Kumari et al., 2022; USEPA, 2004). Once, they enter into the environment especially in soil ecosystems, through oil tanker spillage/leakage, forest fire, coal and fuel combustion, vehicles, road dusting, transportation, pipeline seepage and industrial effluents (Choi et al., 2020; S. Kumari et al., 2018). Industrial effluents are mainly the liquid wastage consisting of various organic and inorganic contaminants like, PAHs, pesticides, heavy metals, dyes etc., from various industries contributing largest contaminants of water bodies and soil. Inefficient public transportation, traffic congestion, and incomplete fuel combustion also contributed to PAHs deposition in the environment.

PAHs often detected in the soil which bears different complex chemical structures. One of the very identified chemical structures is with “Bay” and “K” regions of the phenanthrene with high toxicity (Eldos et al., 2022). These “Bay” and “K”

regions are the area discovered in model compound phenanthrene which show inhibition in interactions with other compounds and due to this compound, it is used to study carcinogenic effects of PAHs. The presence of such regions create site for oxidation and further cleavage of compound during degradation. Hence, regions are important in cleavage initiation of several PAHs compound comprising “Bay” and “K” regions and support during conversion of toxic PAHs into non-toxic carbon compound.

PAHs are multi-fused benzene rings with high molecular weights and hydrophobic compounds that are prone to be adsorbed to the soil particles, and hence their accessibility decreases which result in less bioremediation (Cerniglia & Heitkamp, 1989). Conventional methods for PAHs degradation, such as physical, chemical, mechanical, volatilization, landfilling, and thermal degradation are in use to date. However, these methods have shown adverse effects on the environment as well as on living organisms (S. Kumari et al., 2018; Ping et al., 2017). Recently, various advanced omics technologies like, genomics, metagenomics, proteomics, transcriptomics, and microbial electrochemical systems have emerged as a promising technology for biodegradation of contaminants by exploiting genes and proteins of microorganisms and electrochemically active bacteria (EAB) (S. Kumari et al., 2022; Malik et al., 2022; Pant et al., 2021). However, these technologies have their limitations as it is known that the metagenomics could not reveal the functional expression aspects of bacteria, and to perform the whole metagenomics, transcriptomics, and proteomics experiment and for results analysis required costly materials and instruments which is not always feasible under all conditions (Pant et al., 2021).



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Last few years, several nanoparticles (NPs) are being employed in bioremediation such as metal-oxide, carbon nanotubes, biopolymers, and nanoscale zeolites (S. Kumari & Kumar, 2021; Younis et al., 2020). Modifications in nanocomposites like nano-enabled biochar enhance the porosity as well as surface area of NPs and augment functional groups for specific interaction with PAHs (Linley & Thomson, 2021). Magnetic NPs such as chitosan-coated C18- magnetite have been studied for the remediation of PAHs by absorbing from environment (Zhang et al., 2010). The adsorption characteristic of NPs makes it better candidates for the biodegradation process owing to adsorption specificity and isothermal properties (Yousef et al., 2020).

Microorganisms with combination of NPs could offer better results for remediation purposes due to its unique properties and eco-friendliness to both microorganisms and environment. Therefore, the present work is focused on the deployment of NPs that enhance the remediation of hazardous PAHs contaminated soil. The microorganisms enhanced the degree of PAHs biodegradation using NPs as revealed from several reported studies (El-Sheshtawy & Ahmed, 2017; L. Li et al., 2021). The mechanisms involved in bioremediation, and microbial degradation have assessed more and reflected because of the assistance of dynamic NPs.

Nano-Enhanced Microorganisms Mediated Remediation of PAH Polluted Soil

Nano-remediation technology suggests numerous potentials, particularly PAHs polluted soil remediation and reduced other hazardous pollutants for the safe and clean land and overall environmental benefits. The application of nano-remediation technology greatly improved the water and soil quality by reducing pollutants which results high yield products, vegetation, and enhance survival of indigenous microbes (Rajput et al., 2023). Another environmental benefit of using this technology is that it prohibits the leaching of hazardous compounds from the soil to ground water and limits the contamination. Existing results on NPs functional surface and their connection with biological surfaces make it potential for the remediation of environment (Su, 2017). Remediation of PAHs is difficult through the conventional process; thus, the emergence of different types and concentrations of NPs makes it feasible for the biodegradation of recalcitrant PAHs (Eldos et al., 2022; Rajput et al., 2022). Earlier studies suggested that the biodegradation rate was proven to be enhanced by the application of definite concentrations and types of NPs (Table 1).

Crude oils are one of the major sources of PAHs which are also the main pollutants of soil and water bodies (S. Kumari et al., 2018)). Hence, the merging of nanotechnology with microbial remediation enhances the PAHs remediation potential by several times without producing toxic by-products such as carbon monoxide, metabolites like, 9—fluorenone, dibenz[a,h]anthracene, which inhibits enzymes and cellular activity, and prevents the degradation of other PAHs in the

system during incomplete bioremediation (Muralidharan et al., 2021; Deegan et al., 2022). Biodegradation of crude oils by strain *B. licheniformis* has been reported to be enhanced by the amendment of two different types of NPs such as Fe_2O_3 NPs and $\text{Zn}_5(\text{OH})_8\text{C}_{12}$ NPs (El-Sheshtawy & Ahmed, 2017). The process for the remediation of benzo(a)pyrene (BaP) from the polluted soil by applying silica NPs coated with lipid, 1,2-dimyristoylsn-glycero-3-phosphocholine, bilayers of bacteria (*P. aeruginosa*) proven that the biofunctionalized silica NPs effectively adsorbed PAHs (H. Wang et al., 2015). Further, biodegradation of indeno(1,2,3-cd) pyrene, a six-fused benzene ring has been reported to be enhanced by yeast strain (*Candida tropicalis* NN4) up to 79% in the presence of Fe NPs in 15 days (Ojha et al., 2019).

Similarly, ZnO NPs along with biosurfactants in presence of yeast consortium YC01 was applied for degradation of five fused 50 mg/L BaP. It achieved an enhanced degradation rate of 82.67% in 6 days (Mandal et al., 2018a). Additionally, biodegradation of six fused benzene rings benzo(ghi)perylene (BghiP) by yeast consortium YC04 has been enhanced up to 63.8% in presence of ZnO NPs after 6 days (Mandal et al., 2018b). Strains of yeast consortium YC01 and YC04 with ZnO NPs have been established as a potential candidate in degrading BaP and BghiP of such a high concentration only in 6 days compared to earlier studies reported. Moreover, magnetic NPs are not only reported to enhance the remediation process, but also aids as a beneficial tool for the successful isolation of PAHs degrading bacteria like *Pseudomonas* and *Sphingobium* sp., from the contaminated sites (J. Li et al., 2018). Modification of the electrode surface by input of magnetite NPs enhanced the efficiency of bioremediation of PAHs in polluted soil in the electrokinetic system (Pourfadakari et al., 2019). The electrokinetic system also referred to microbial electrochemical systems (MES) which influence the rate of degradation of PAHs. The mechanism of MES explains that EAB oxidized the PAHs from the soil at cathode in MES chamber and forward the reaction toward the anode where electricity is generated in the chamber at the real time of biodegradation.

A detailed discussion was reported on advances in bacterial and fungal enzymes immobilized in materials at the nanoscale are effective and capable of being preserved without causing any harm to the environment for environmental clean-up (Gao et al., 2022). Extraction of specific enzymes from indigenous microorganism's cells and immobilization with NPs are reported to actively execute the enzymatic conversion of PAHs, hence, the potential for bioremediation of hazardous PAHs. Several microbial enzymes like manganese peroxidase (MnP), lipase, laccase, catalase, etc. were studied to be immobilized in respective NPs such as nanoclays, Mn NPs, and TiO_2 NPs for effectively mitigate PAHs, and the enzymes seems to be stable even under adverse situations for instance, at different pH range like 5.0 to 8.0 and 4.5 to 8.0, temperature between 30°C to 70°C and 50°C to 60°C, and long storage of 14 and 20 days

Table 1. Nano-Enhanced Degradation of PAH Mediated by Microorganisms.

S. NO.	MICROBIAL SPECIES/ CONSORTIUM	PAHS/CRUDE OIL	NANOPARTICLES (NPS)	DEGRADATION (%) NPS PRESENCE/ABSENCE	REFERENCES
1.	<i>Mycobacterium vanbaalenii</i> PYR-1	Phenanthrene	Single-walled carbon nanotubes	59.5%/42.4%	Cui et al. (2011)
2.	<i>Micrococcus lutes</i> RM1	Pyrene	MFe ₃ O ₄	-	Saed et al. (2014)
3.	<i>Pseudomonas aeruginosa</i>	Benzo(a)pyrene	Silica NPs	-	H. Wang et al. (2015)
4.	<i>P. mendocina</i> H3, <i>P. pseudoalcaligenes</i> H7, <i>P. stutzeri</i> H10, <i>P. alcaligenes</i> H15, <i>P. pseudoalcaligenes</i> H16, <i>P. mallei</i> 36K and <i>Micrococcus luteus</i> 37	Crude-oil	Nano-carbonized rice husk	-	B. Kumari and Singh (2016)
5.	<i>Bacillus licheniformis</i>	PAHs-crude oil	Fe ₂ O ₃ NPs, Zn ₅ (OH) ₈ C ₁₂	-	El-Sheshtawy and Ahmed (2017)
6.	<i>Rhodotorula</i> sp. NS01, <i>Debaryomyces hansenii</i> NS03 and <i>Hanseniaspora valbyensis</i> NS04.	Benzo[ghi]perylene (BghiP)	ZnO NPs	63.83%/60%	Mandal et al. (2018b)
7.	<i>Bacillus cereus</i>	Phenanthrene, anthracene	Graphene oxide	52.7%	Mu et al. (2019)
8.	<i>Candida tropicalis</i> NN4	Indeno(1,2,3-cd) pyrene	Fe NPs	79%/61%	Ojha et al. (2019)
9.	microbe consortium	PAHs	Ag ₃ PO ₄ @Fe ₃ O ₄	49.83%	C. Wang et al. (2019)
10.	<i>Rhodococcus opacus</i>	PAHs	nano-biochar	-	Goswami et al. (2020)
11.	<i>Methanosarcina</i> and <i>Methanosaeta</i> , <i>Pseudomonas</i> , <i>Clostridia</i> , and <i>Synergistetes</i>	Pyrene	FeS or magnetic carbon	77.5% and 72.1%/40.8%	L. Li et al. (2021)
12.	<i>Pseudomonas stutzeri</i> NA3	Anthracene, pyrene, and benzo(a)pyrene (BaP)	Fe NPs	85%	Parthipan et al. (2022)

(Acevedo et al., 2010; Chronopoulou et al., 2011; Hou et al., 2014).

It is explored that the nano-sized zero-valent iron (nZVI) can effectively participate in the biodegradation of persistent environmental pollutants (Benjamin et al., 2019; Galdames et al., 2017). nZVI NPs are most often used due to its size and high reactivity to a contaminant such as PAHs. The bacterial strains such as *P. stutzeri* NA3 and *Acinetobacter baumannii* MN3 enhanced degradation of mixed PAHs (anthracene, pyrene, and BaP) by 85%, increases in bacterial biomass, improved PAHs availability, and adsorption of PAHs on the surface of iron NPs (Parthipan et al., 2022). Thus, NPs reflect a promising candidate for the nano-remediation of environmental soil PAHs (Binh et al., 2016).

Several microorganisms particularly, bacterial species such as *Geobacillus stearothermophilus*, *P. aeruginosa*, *B. subtilis*, and fungal species, like *Fusarium oxysporum* are actively synthesized Au, ZnO, and Fe-based NPs which employed in remediation of environmental persistent pollutants (Mukherjee et al., 2002; B. N. Singh et al., 2014; Sundaram et al., 2012). These NPs are

proven to be more effective and more stable than chemically synthesized NPs (Kapoor et al., 2021) and getting more scientific attention. It provides excellent advantages in comparison to chemical synthesis with similar property, due to achieving large surface to volume ratio, improved catalytic performance, eco-friendly, time saving, sustainable, quick, and low-cost. The representative means of nano-remediation of PAHs contaminated soil using both NPs and bacteria has been showed in Figure 1.

Mechanism of NPs-Microbes Interaction in PAHs Degradation

Microbial interaction with NPs to adsorbed PAHs is complicated due to its complex molecular structures and studies related to its mechanism are not very clear. Several studies tried to explain the possible mechanisms involved in PAHs degradation which varies from one strain to other and NPs property (El-Sheshtawy & Ahmed, 2017; Kapoor et al., 2021; Parthipan et al., 2022). The possible mechanism is that bacteria secrete specific enzymes get immobilized in NPs on which PAHs get

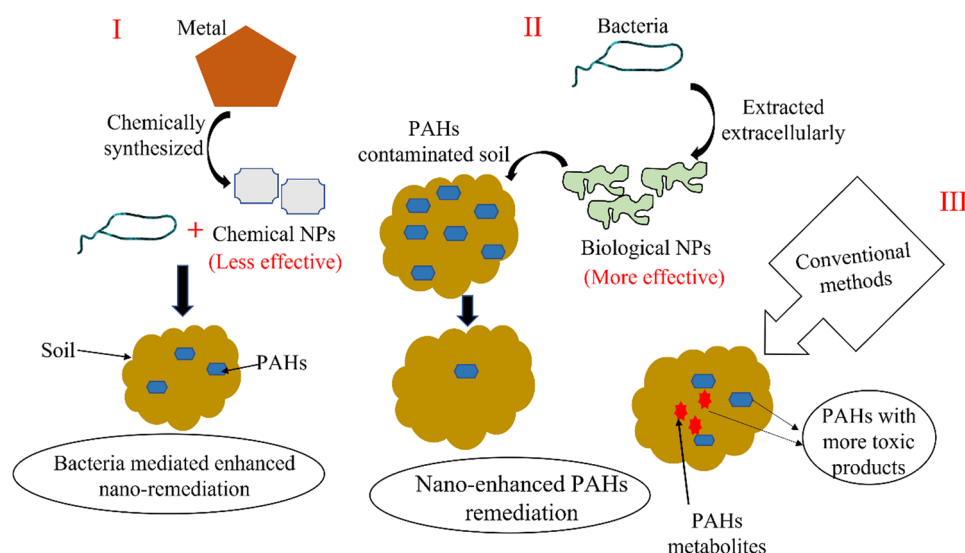


Figure 1. Different representative modes of enhanced nano-remediation of PAHs contaminated soil deploying nanoparticles and bacteria.

adsorbed and mediate degradation or bacteria can extracellularly degrade PAHs efficiently. The types of interaction and intensity of microorganisms and NPs could be important factor that enhanced persistent compounds biodegradation (J. Singh et al., 2019). NPs interaction with compounds and microbes has been highlighted with the support of reported studies. The structure of graphene oxide quantum dots is like aromatic hydrocarbons which are specific to isolate *B. cereus*, a PAHs degrading bacterium, from polluted soil and further enhance biodegradation. It was found that graphene oxide quantum dots stimulated bacterial proliferation and PAHs degradation, even after the 20th generation. The effects were persevered without further exposure to graphene oxide quantum dots, and transformation occurred in bacteria was transferred to the next generation (Mu et al., 2019).

In addition, the S-layers and lipopolysaccharides (LPS) present in the bacterial cells make feasible for attachment of NPs-sorbed-contaminants and bacteria. The LPS consist of lipid, core polysaccharides and o-antigen. The composition of LPS sugar affects the formation of biofilm (Abdel-Rhman, 2019), that is formed by aggregation of diverse bacteria which consist of various components like, extra polysaccharide substance (EPS), DNA, proteins etc., which assist to adhere with solid substratum. It is reported that the biofilm could enhance the PAH biodegradation and is important phase of bacteria and the biofilm formation enhance by inhibiting its flagellar gene transcription results in bacterial non-motility (Lahiri et al., 2022). The electrostatic interaction of anionic and cationic between LPS and NPs play an important role. J. Singh et al. (2019) reported with evidence that NPs along with the adsorbed phenanthrene compound extracellularly attached to the bacteria. This would enhance the uptake of compounds and increase bacterial conversions. In Figure 2, mechanism of how NPs-sorbed-PAHs gets access to bacteria and bacterial

enzymes immobilization takes place which ultimately enhances PAHs biodegradation by providing a change in structure related to a large surface area and pores are shown.

Nowadays, molecular mechanism overcome the issue of less stability of NPs structure and synthesized the green NPs with controlled dimensions. For green synthesis, nitrate reductase is known to be a major reducing agent and stabilizer. Green synthesis is an eco-friendly approach for the synthesis of metallic NPs by using suitable solvents, diverse plant extract or phytochemicals (terpenoids, flavonoids, etc.) and microorganisms like fungi, bacteria, etc. The major advantage of the method is the limited production of waste products, highly energy saving process, cost effective and is applicable for large scale production (J. Singh et al., 2018). There are multiple cellular machineries are involved in the biological synthesis of NPs (Ali et al., 2016, 2017).

The enzymes (nitrate reductase), electron shuttle, and cofactors (NADPH) of bacteria, *B. licheniformis*, are responsible for the reduction of ionic Ag into Ag NPs (Vaidyanathan et al., 2010). Whereas, not in all but in case of fungus (*F. oxysporium*), the synthesis of Ag NPs has been reported due to the contribution of catalytic protein, NADH-dependent reductase. The stability of NPs is provided by the external addition of phytochelatin and 4-hydroxyquinoline (Ali et al., 2019; Durán et al., 2011). Some mechanisms related to the interaction between NPs structure, contaminants and microbial outer surfaces are still needed to explore with the evidence of experimental support. In nano-bioremediation, adsorption is the crucial procedure. The surface and pores of NPs on which PAHs get adsorbed and makes them accessible to the microorganism for degradation is the key reason (Park et al., 2003; Xia & Wang, 2008). Other mechanism used adsorption kinetic models that explain the adsorption of PAHs are pseudo-first and second-order models (Revellame et al., 2020). The other

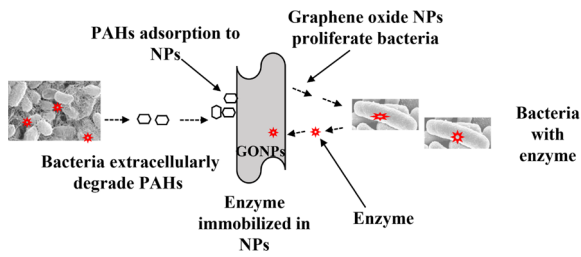


Figure 2. Mechanism of PAHs degradation by bacteria via extracellularly and enzyme immobilization in graphene oxide nanoparticles (GONPs).

mechanistic model is the adsorption isotherm which clarifies how much contaminants get adsorbed to the adsorbent, and are expressed by the Langmuir model, Freundlich model, Sips model and Temkin model (J. Wang & Guo, 2020).

The use of NPs is a remarkable technique with impactful results in bioremediation and this could be achieved by proper modification of material surface by chemical and physical alteration during synthesis. Some critical tasks that must be considered while developing NPs for remediation purpose are formulation process at nanoscale, non-toxicity, green chemistry, cost effectiveness, biodegradability, and recyclability. Hence, it become necessary to understand the correct way of fabrication process, nature of materials and stability in environmental conditions to limit the adverse effects on soil environment (Guerra et al., 2018).

Concluding Statements

With the growing need for conveniences as well as getting rid of toxic pollutants, the urgent requirement for innovative, eco-friendly, effective methods which enhance biodegradation of PAHs without hindrance is of utmost importance. Recently, the nanotechnological approaches ensure the enhanced remediation process without disturbing the environment. The input of NPs in soils enhance microbes or agriculturally important microbes and their functionality to improve biodegradation of pollutants. NPs make an exceptional impact on remediation process by stimulating microbiological activity after combination with microbial responses. However, the aspect is not well explored such as how nanotechnology could improve PAHs degradable microbes and its functionality as limited studies are available. Thus, it is required to have large number of laboratory and field experiments to understand the exact role of NPs to enhancing microbial community and remediation process especially various types of unfavorable environment. Better understanding regarding the soil biota coupled with the ecological NP's behavior could ensure the safe use of NPs as a nano-enhanced bioremediation. The fate of NPs in realistic environment is concerning issue as not yet fully explored, and the input of NPs in nano-enhanced microbial remediation of PAHs contaminated soil should be carefully implied. However, current critical evaluation of nano-enhanced microbial remediation approaches could be helpful to exploring insights realistic application of NPs in soil pollutant clean-up program.

CRedit Author Statement

Vishnu D. Rajput, and Smita Kumari: Conceptualization, Methodology, Software Vishnu D. Rajput, Smita Kumari: Data curation, Writing- Original draft preparation Vishnu D. Rajput, Smita Kumari.: Visualization, Investigation. Tatiana Minkina, Svetlana Sushkova, Saglara Mandzhieva: Supervision.: Tatiana Minkina, Svetlana Sushkova, Saglara Mandzhieva: Software: Writing- Reviewing and Editing, Vishnu D. Rajput, Smita Kumari.

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Consent for Publication

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