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Techniques of Bioremediation using bacteria for the treatment of polycyclic aromatic hydrocarbons: A Review

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ABSTRACT

The term "hydrocarbon" is self-explanatory and refers to solely carbon and hydrogen compounds. Hydrocarbons play an important role in our everyday lives. Hydrocarbons, particularly polycyclic aromatic hydrocarbons, harm biota. The relatively fast introduction of xenobiotic compounds, as well as the enormous movement of natural materials to various environmental compartments, can often overwhelm the self-cleaning capabilities of the recipient ecosystem, resulting in pollution and accumulation of hazardous or even lethal levels. Bacteria capable of hydrocarbon degradation are frequently used in the bioremediation of fuel oil-contaminated sites. Presently, multiple sophisticated methodologies, transcriptomics, proteomics and are effectively utilized for the depiction of hydrocarbons degrading microorganisms. These expertises are highly developed, and its integration with bioinformatics tools makes it even more efficient. Though health science and biological science are the major relevant areas for molecular docking, it has been effectively used to explore the process of bio-degradation in ecological remediation in recent years. This review focuses on the sources, fate of PAHs, human exposure, various computational aspects associated with PAHs, and some approaches of synthetic biology related to pollutant degradation and PAH-degradation by genetically engineered microorganisms.

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1 Introduction

Now in these days pollution related to hydrocarbon is the most serious concern. Various factors like waste disposal, unintentional spills, pesticides, and losses during shipping and storage, are some ways through which hydrocarbons come into the environment. Unfortunately, oil spills are seriously associated with the hydrocarbon contamination of agricultural fields, which kills vegetation and the biodiversity connected with it (Mishra 2020). PAHs are a type of chemical compounds that are usually colorless, pale yellow, or white in colour. PAHs are a family of chemicals that are found in almost every environment and consist of hundreds of chemically related compounds. These chemicals can survive in the environment for many years and have a wide range of structures and detrimental effects (Patel et al. 2020). Further, these chemicals have demonstrated a variety of negative health impacts on the human system. Hydrocarbons are the most frequently used energy and fuel resources on the planet because of the energy they create. Spills that appear to be unavoidable during ordinary operations of crude oil production, refining, and distribution, as well as a result of severe accidents, have aroused interest in this sector (Patel et al. 2020). Since during oil field incidents, leak from oil pipes and storage space reservoir, oil tanker, and seepage calamities, and renovations of refineries and petrochemical fabrication apparatus are all widespread causes of petroleum hydrocarbon spills and discharges (Petrov 2012).

Despite the difficulty of treating oil pollution, microorganisms that can degrade petroleum hydrocarbons have progressed as a consequence of living in close contact with biologically occurring environmental petroleum hydrocarbons. These organisms could be used to clean up oil pollution (Lea-Smith et al. 2015). Therefore identification of the bacteria which can digest left-over products from the food, farming, biochemical, and medicinal industries is a necessary step of bioremediation. Due to less cost and eco-friendly character, the usage of bacteria to contract environmental toxins has become a potential tool in today’s time (Guerra et al. 2018). The advancement of molecular tools, as well as a piece of improved knowledge about microbial metabolic and genetic organizations and activities, has expedited the spread of recombinant DNA engineering strategies to improve bioremediation for the elimination of contaminants from the atmosphere. Molecular docking is a simple and low-priced method for accurately understanding the bioremediation of the PAH mechanism of enzymes or proteins through Ligand. The present review article aimed to study the mechanism of PAH degradation by some recent approaches of synthetic biology related to pollutant degradation and PAH-degradation by genetically engineered microorganisms (Figure 1).

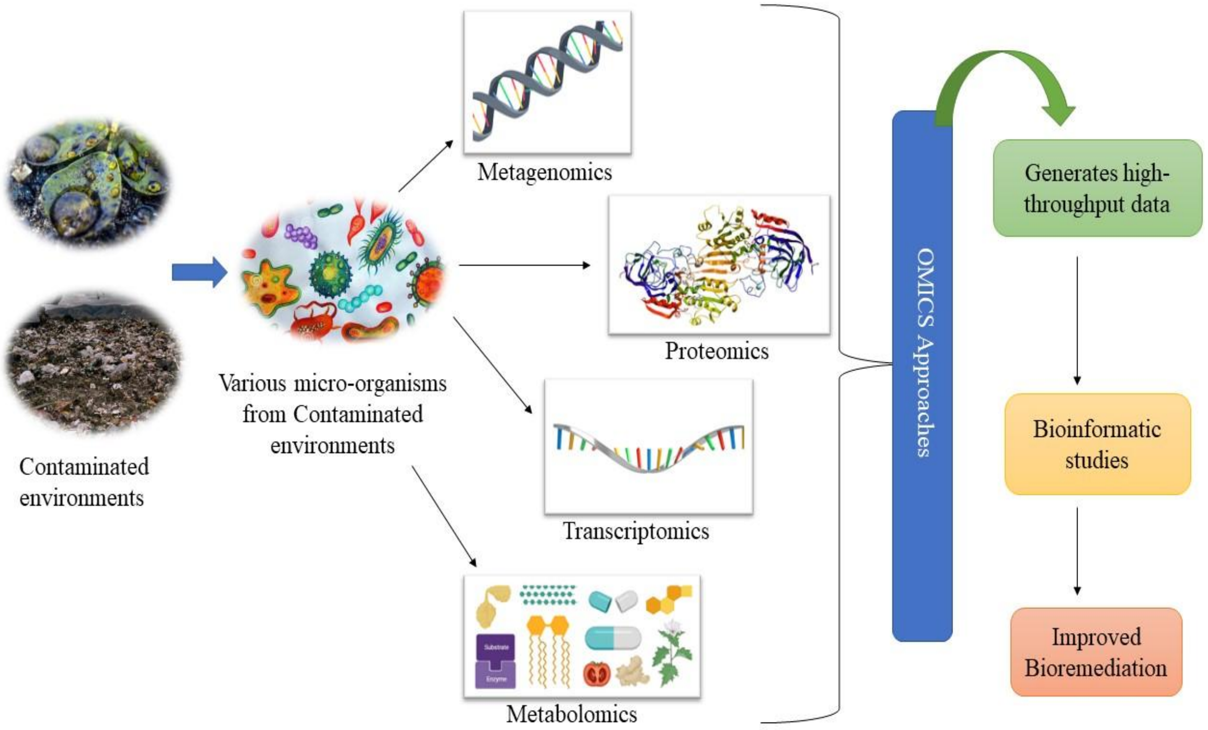

Figure 1 Graphical representation of an integrated strategy for biodegradation of xenobiotic substances using sophisticated technologies (Tanveer et al. 2018).

1.1 Hydrocarbons

Oil production and shipping cause accidental contamination of petroleum hydrocarbons in the soil. Every year, approximately 8.8 million metric tonnes of oil are spattered on land (Jawaid 2022). These hydrocarbons might be carcinogens or neurotoxic organic pollutants when they are available in higher quantities. Hydrocarbons pollute the soil, air, and freshwater (both surface and groundwater), particularly polycyclic aromatic hydrocarbons (PAHs), which have sparked a public concern since many PAHs are lethal, mutagenic, and carcinogenic (Shukla et al. 2022). The toxicity of PAHs has been reported to increase with increasing molecular weight and alkyl substitution in the aromatic ring (Jesus et al. 2022). The hydrocarbons reach horizontally on the surface of groundwater and contaminate it extensively. The lower boiling-point portions, particularly those in the C10–C19 range, have a strong correlation with petroleum hydrocarbon toxicity. Aromatic, aldehydes, ketones, carboxylic acids, fatty acids, etc. were among the metabolic components that led to the biota toxicity of petroleum pollution. Earthworm and seed germination assays can be used to assess the toxicity of organically contaminated soil (Tang et al. 2012). TPH (total petroleum hydrocarbon) is an intricate combination of substances that includes saturated hydrocarbons, aromatic chemicals, asphaltenes, and resins. High quantities of total petroleum hydrocarbons (TPH) in the severely polluted soil impeded seed germination and created nutrient-deficient circumstances in which the plants could not survive. For successful rhizo-degradation, certain soil environmental variables (e.g., oxygen permeability and water availability) must be increased (Haider et al., 2022). The major sources of hydrocarbon pollutants are crude oil, used engine oil (spent oil), and diesel.

1.2 The Elementary components of Hydrocarbons

Polyaromatic hydrocarbons degrading bacterial microbes are using enriched naphthalene (20 isolates), phenanthrene (25 isolates), or anthracene (6 isolates) as the sole carbon and energy source (Mangwani et al., 2021). Three isolates, N6, A3, and P3, that were found to use naphthalene, phenanthrene, and anthracene better than the other isolates, were subjected to additional characterization (Al-Thani et al. 2009). Cycloparaffins are a solitary cause of carbon that has proven to be ineffective. The coordinated occurrence of a diverse microbial population on cyclohexane has been revealed, implying that commensalism is involved in the microbial degradation of cycloparaffinic hydrocarbons (Sime-Ngando et al. 2018). Microorganisms are a very dependable approach to biodegrade petroleum-derived materials. Even *Bacillus subtilis* inoculum completely converts the synthetic and semi-synthetic vehicle lubricant oils and changes the color of DCPIP (2,6-Dichlorophenolindophenol) from blue to translucent (Bidoia et al. 2010). According to a study on the isolation of *Cladosporiunz resinae* from soil and air samples, it was reported that *C. resinae* strains can easily develop a deep culture on C10 to C14 n-alkanes. Other strains thrive on alkanes ranging from C9 to C18 (Jun et al. 1973).

2 PAHs and their sources

PAHs are obtained from a variety of sources, including human activities as well as natural sources. PAHs are formed primarily as a result of thermal alteration, incomplete burning of organic matter, combustion of fossil fuels, and industrial wastewater. Natural sources of PAHs include volcanic eruptions, oil seeps from crude oil reservoirs, bush burning, and erosion of ages' sediments, whereas anthropogenic sources include thermal alteration, incomplete burning of organic matter, combustion of fossil fuels, and erosion of ages' sediments (Okoro et al. 2020). Petrogenic, pyrogenic, and biological methods are the three main ways by which PAHs are delivered into the environment. Petrogenic PAHs are those that are generated during crude oil preparation and other related events. Petrogenic PAHs are formed primarily by petroleum products and are the most common sources of PAHs due to the widespread use of various modes of transportation, as well as the widespread storage of crude oil and its products in the environment. The major sources of petrogenic PAHs are oil spills from marine and freshwaters, tank leaks from petroleum products stored beneath and above ground, and the retention of various quantities of discharge of gasoline, motor oil, and other substances. Pyogenic PAHs are produced during pyrolysis. Pyrolysis is the non-oxygenated putrefaction of organic matter at elevated temperatures (350°C to >1200°C). The harsh condensation of coal into coke and coal tar, as well as the heated splitting of oil by-products into smaller hydrocarbons, are common examples of pyrogenic sources of PAHs that exist naturally in the environment. Inadequate combustion of fuels in automobiles and trailers, partial burning of wood in forests, and a variety of other pyrolysis processes contributed to the creation of PAHs. Biologically produced PAHs are produced from a range of biological activities, as well as the bio-synthesis of some plants, microorganisms, as well as plant breakdown (Hussein et al. 2016).

3 Bacterial degradation of Polycyclic aromatic hydrocarbons

The removal of PAHs from the environment has been studied using a variety of bioremediation methods during the past 30 years. In terms of PAHs decomposition, bacteria have shown the best and most economical methods when compared to fungi, algae, and plants. Bacteria have evolved, developed, and adopted a technique to digest several environmental contaminants for more than three billion years (Ghosal et al. 2016). Numerous researchers have also established many metabolic pathways in bacteria that are involved in the breakdown of PAHs (Govarthanan et al. 2020). The majority of the time both aerobic and anaerobic conditions are used for the bacterial-based PAHs breakdown techniques. Oxygen, which also

Table 1 Bacteria and their respective degradable PAH

PAH degraded	Bacteria	References
Phenanthrene	*Pseudomonas, Acinetobacter, Arthrobacter, Mycobacterium,*	Li et al. 2021
Pyrene	*Rhodococcus, Bacillus, Xanthomonas, Mycobacterium, Pseudomonas*	Shahsavari et al. 2019
Naphthalene	*Acinetobacter, Alcaligenes, Pseudomonas, Sphingomonas*	Rabani et al. 2022
Fluoranthene	*Pseudomonas, Arthrobacter, Mycobacterium*	Fathepure 2014
Anthracene	*Alcaligenes, Rhodococcus, Beijernickia, Mycobacterium, Sphingomonas, Janibacter*	Shahsavari et al. 2019

acts as a co-substrate for the aromatic compounds' hydroxylation and oxygenolytic ring cleavage, is the final electron acceptor in the aerobic type of PAH breakdown. The bacteria in anaerobic PAHs degradation take completely different routes to break down the aromatic ring based on reductive processes (Mallick et al. 2011).

The aerobic mode of PAHs degradation has received the greatest attention from researchers for several decades, but in recent years interest in the research on the anaerobic technique have been increased. The majority of anaerobic catabolism occurs in aquatic sediments, submerged soils, and aquifers, where ferric ions, sulfate, and nitrate serve as the bacteria's ultimate electron acceptors (Foght 2008). Additionally, the aerobic environment is favorable for the breakdown of PAHs as demonstrated by oxygenase-mediated metabolism carried out by monooxygenase or dioxygenase enzymes. The aerobic decomposition of PAHs is the hydroxylation of an aromatic ring, which is carried out by the action of two enzyme’s dioxygenase and dehydrogenase, and these work on a cis-dihydrodiol and rearomatized to a diol. Further, cleavage of these diol intermediates by extra- or intra-diol ring-cleaving dioxygenases via the ortho- or meta-cleavage mechanism produced catechols by the TCA cycle (Mallick et al. 2011). Numerous studies have demonstrated how effectively certain bacterial species can use PAHs as their only carbon source.

Employing fungi and bacteria under a co-cultivation technique for PAHs biodegradation is more promising than employing monoculture. According to Arun et al. (2008), significant outcomes demonstrating the value of co-cultivation were seen when *Basidiomycetes* fungus and *Pseudomonas* spp. were co-cultured. *Coriolus versicolor* and *Fomitopsis palustris* co-cultivated with *Pseudomonas* spp. also demonstrated 93.7% effectiveness in the degradation of pyrene (Table 1).

4 Polycyclic aromatic hydrocarbons: computational aspects

4.1 Bioinformatics

Bioinformatics fostered a modern set of computational tools that combines information technology (IT) with biology. This cutting-edge technology collects data from a variety of high-throughput biological systems and stores it all, allowing researchers to explore and regulate the association between organic molecules such as biochemical and metabolic conduits, expression of proteins, structures, metabolites, and macromolecular sequences structures (Shekhar et al. 2020). Protein, DNA, and RNA sequences provide enormous volumes of information that must be carefully carried out; as a result, bioinformatics has resulted in the creation of specialized computing tools to assess such enormous amounts of biological data (Bhatt et al. 2021). As a result, bioinformatics-related technologies are critical for understanding harmful pollutant bioremediation. The molecular, genetic, and cellular origins of xenobiotic breakdown and detoxification are better understood using bioinformatics (Huang et al., 2020).

Bionemo, a database created by the Spanish National Cancer Research Center's structural computational biology department, provides information on particular genes and proteins involved in the bio-degradation reaction and metabolic routes (Carbajosa et al. 2009). Unified bioinformatics-based approaches are used to uncover the inherited and functional characteristics of unlike soil microbial populations utilizing MetaPhlAn, LEfSe, XLSX, and KEGG bioinformatics databases for metagenomics-based categorization of soil microbial populations of diverse soil sites (Kumar et al. 2016). Degradation of contaminants by microbial populations is a favorable potential and suitable remediation skill, and there is no specific source available that contains all of the data about environmental toxins, microorganisms, and their bioremediation capabilities. As a result, these databanks combining comprehensive insight into the type of contaminants, their enzymes, metabolic processes, catabolic genes, and protein-expression reports can be a valuable tool for enlightening upcoming investigations in the area of bioremediation.

4.2 Quantum mechanical techniques

The researchers gathered PAHs that interact with NDO and their reaction outcomes in a systematic manner. The relevance of in silico bioremediation through DFT investigations signifies that thermodynamically all the polycyclic aromatic hydrocarbons can associate with the active binding sites of NDO. Only steric hindrance governs whether compounds (enzymes and PAHs) can optimally react with NDO or not. Some researchers used the exact relevance of the structure/reactivity correlation to enhance the understanding of PAH-enzyme interactions (Librando and

Alparone 2007). The PAH degradation efficiency was predicted using a combination of ab initio and functional density theoretical models for a range of dimethyl naphthalene (DMN) isomers. These findings aid the idea that electronic polarisability, in addition to performing a crucial task in the biodegradation of DMNs and stipulating a foundation for Farnet's hypothesis, could be an effective method for calculating the biodegradation tendencies of a set of molecules. The approach was integrated with docking procedures to successfully illustrate PAH–NDO connections.

The process of PAH-enzyme interactions is not entirely understood, and experimental procedures are unlikely to reveal it. Recent research using a quantitative structure activity relationship (QSAR) methodology implies that some investigational methods could be very useful in this regard (Ha et al. 2019). The binding relationships between PAHs and diverse compounds such as DNA or oestrogen receptors were explored by Li et al. (2012). The binding action was connected to van der Waals volume, molecular size, shape outline, hydrogen bonding, polarisability, hydrophobicity, electron topological state, and p–p interactions, according to the descriptors used in the QSAR models (Gbeddy et al. 2020). In these investigations, QSAR has been used to assess for mutations that progress enzyme activity. Furthermore, QSAR was also used to give proper details regarding the toxicity of PAHs and degradation arbitrates (Kobeticova et al. 2011), revealing that toxicity and lipophilicity (Kow) have a significant relationship, implying that non-polar narcosis is the most common lethal consequence of the tested PAHs. This is since toxicity, which is specifically linked to hydrophilicity for biological membranes (i.e., non-polar narcosis), is typically determined by the quantity of the molecule accumulating in the same membranes.

4.3 Database approach

The massive volume of information on the interaction between the environment and degradation makes it difficult to discover the proper reaction, degradation products, and so on. As a result, a database strategy could be beneficial. The incorporation of different sources of bioremediation information is one problem connected with the database strategy. In this regard, Pazos et al. (2005) provided a fine-tune 'metarouter,' which is a valuable tool for evaluating the ecological future of compounds and creating bio-derivative methods for such groups. It provides information regarding the name, molecular weight, the image of chemical structure, chemical formula, SMILES code, canonical 3D structure in PDB format, physicochemical properties, and connections to other databases are provided for chemical compounds. For reactions, the following information is provided: substrates, products, catalyzing enzymes, and relationships with other databases. The drawback of metarouter's is that it concentrates on the biochemical features of biodegradation instead of the type of biomolecules that perform the processes. Carbajosa et al. (2009) presented Bionemo, a novel database that complements current biodegradation databases, in a recent study. Bionemo was created by integrating data (manually) from available articles and the biodegradation literature in general into a fundamental biochemical network. In addition to transcription regulation information for more than 100 promoters and transcription factors, Bionemo now offers sequence data for 324 processes. Meanwhile, current biodegradation databases tie reactions to protein sequences in databases identified by EC codes. This strategy, however, may be inappropriate. Many reactions, for example, have the same EC code even though they utilize various substrates and produce numerous products. Bionemo is a databank that includes metabolic, genetic, and regulatory data. Enzymatic complexes are the database's principal entries. These are associated with biological reactions that convert substrates to products.

4.4 Docking studies

PAHs such as anthracene, phenanthrene, pyrene, and benzopyrene are made up of two or more benzene rings and include both hydrogen and carbon. PAHs are also characterized as electroneutral, non-polar compounds retrieved from oil and coal that lack auxiliary branching substituents on their ring structures. Inadequate combustion of wood, coal, oil, tobacco, and organic polymer mixtures produces them. These are major pollutants in the environment as well as a source of food contamination. Although physical and chemical approaches can be used to adsorb, decompose, and attenuate PAHs but these are associated with some environmental issues like air, soil, and groundwater pollution (Xu et al. 2012). Biodegradation as an alternative approach might be a highly efficient way to convert PAHs into H_2O and CO_2 (Ukiwe et al. 2013). Biodegradation is aided by a variety of microorganisms. The mechanisms of degradation have previously been discovered at the molecular level. However, there is a need to put attention to receptor-ligand interactions from an enzyme or protein standpoint (Jin et al. 2015). The efficiency with which PAHs are removed varies from species to species.

In an attempt to improve degrading potentials, the mutants could increase the formation and lessen nuclear repulsion by simply changing steric interactions (Librando and Pappalardo 2014). Similarly, while addressing fluoranthene breakdown and bond structure based on the active site of ring-hydroxylating dioxygenase in *Microbacterium paraoxydans* JPM1, researchers found the fluoranthene molecule was surrounded by hydrophobic residues. Two oxygen atoms and a mononuclear iron atom formed a triangle with the terminal of Asn207, forming two hydrogen bonds.

Even though the number of benzene rings has an impact on the bonding energy, the width/height ratio of the substrate is also an important physical characteristic. When molecules are afar than the

Table 2 Various docking software and their algorithms

Software	Algorithm	References
PythDock	Particle swarm	Chung et al. 2011
MolDock	Guided variance evolution	Thomsen and Christensen 2006
GOLD	Genetic	Verdonk et al. 2003
Molegro Virtual Docker	MolDock SE	Storn and Price 1997
AutoDock	Lamarckian Genetic	Morris et al. 2009
FlexX	Incremental creation	Schellhammer and Rarey 2004
Surflex-Dock	Molecular resemblance-based search	Jain 2007
AutoDock Vina	Broyden-Fletcher-Goldfarb-Shanno	Trott and Olson 2010

width/height ratio threshold, they have complexity in interacting alongside the active site of the enzyme. Various docking software are used for the biodegradation of PAHs, information regarding the available docking software is available in table 2.

5 Synthetic Biology-Based Alternatives for Microbial Remediation of PAHs

5.1 Genetic and Metabolic engineering

The term "genome editing" was proposed by Enrquez (2016) and it is used rational genetic manipulation at a local (gene) or global (genome) level to assure precise addition, removal, or substitution of DNA fragments. Transcription activators such as zinc finger nucleases and effector nucleases (TALEN) are mostly used in this gene editing. According to Kanchiswamy et al. (2016), CRISPR_Cas is the most proficient and reliable method for gene editing methods. Genome editing helps in accelerating the rate of the bioremediation process. Further, TALEN is a sequence-specific DNA-binding component that is specific to the particular host genome (Utturkar et al. 2013). When TALEN attaches to DNA, it worked on the target sequence and creates sticky ends, and produced DSB (Double-stranded break) in the target sequence. On the host genome's target site, the Fok1 cleavage domain produces DSBs. Another method of using hybrid nucleases made up of TALENs and ZFNs nucleases was devised to address the molecular complexity. On the other hand, the CRISPR-Cas system offers distinctive sequence specificity and sophisticated gene editing as unique action properties. These gene-editing methods produce knock-in and knock-out effects and are currently being tested in bioremediation investigations (Kumar et al. 2018). According to the latest details, the CRISPR-Cas system is primarily used and executed by scientists working with ideal organisms such as *Pseudomonas* (Nogales et al. 2020) or *Escherichia coli* (Chen et al., 2018). In the field of bioremediation, new insights into CRISPR toolkits and creating gRNA for the manifestation of function-specific genes relevant to remediation in non-model organisms (e.g., *Comamonas testosteroni*, *Rhodococcus ruber* TH, and *Achromobacter* sp. HZ01) are also being proposed (Liang et al. 2020). Pollutant-inhabited bacteria are the best applicants for gene editing and metabolic engineering since they are acclimated to surviving and harboring in the presence of diverse toxic and non-degradable xenobiotics. Furthermore, interpreting metabolic pathways appears to be valuable in exploring microbial bioremediation (Plewniak et al. 2018), such as the decontamination of pyrethroid from the soil using the fenpropathrin biodegradation pathway studied in *Bacillus* sp. DG-02 and the bioremediation of hazardous pollutants via the manufacturing pathway of haloalkane dehalogenases (Chen et al. 2014). Metabolic engineering involves modifying an existing route to improve the bioremediation method (Michel et al. 2007). This strategy chiefly wraps the analysis of microbial enzymes, i.e., oxidases, oxidoreductases, esterases, phenoloxidases, and monooxygenases participating in diverse degradation stages. Enzymatic bioremediation is a simple, quick, and ecologically favorable approach for microbial removal and decomposition of relentless xenobiotics (Sharma et al. 2018). With the limitation of lower productivity, isolation and characterization of microorganisms by enzymatic potentials have been carried out. Organophosphates (OP) and organochlorines (OC), two of the main components of pesticides, accumulate in agricultural soil and get into water bodies through agricultural runoff (Panelli et al., 2017). It has been demonstrated that genetically altered bacteria can efficiently bioremediate methyl parathion (OP) and hexachlorocyclohexane (OC) (Gong et al. 2016). Additionally, using genetically altered *P. putida* KT2440, organophosphate, and pyrethroid bioremediation has been carried out (Zuo et al. 2015). The catabolism and destruction of numerous persistent substances have been observed since the advent of metabolic engineering.

5.2 Artificial Genetic Circuit and Microbial Biosensor

The insertion of the artificial genetic circuit necessitates the use of chassis. The Recombinant DNA Advisory Committee has designated *P. Putida* as the Host Vector Bio safety strain. It's GRAS (Generally recognized as safe) when it comes to environmental discharge. Because it had a high tolerance to fluctuating circumstances such as pH, temperature, toxins, solvents, osmotic, and oxidative stress, it is excellent to be used for the future creation of synthetic biology framework panel. *P. putida* also has a flexible metabolism and minimal nutritional prerequisites (Pabo and Nekludova 2000). These characteristics formulate this organism as the ideal bacterial prototype for bio-remediation purposes in the environment (Tanveer et al. 2018). Recently, a synthetic genetic circuit was created for *P. putida*, which has specific promoter genes for persistent chemical degradation, and the expression of these genes depends on the available pollutant (Adams 2016). For the construction of synthetic genetic circuits, serine integrases were used. Microbial cells benefit from the processes of the biological system that regulate cell growth and responses to environmental stimuli such as light, temperature, pH, and oxygen (Tropel and Van Der Meer 2004). The amounts of various persistent chemicals present will affect how microbes living in the surrounding environment at a contaminated site respond. Whole-cell biosensors for detecting the occurrence, and biodegradation ability of xenobiotic chemicals (paraffin, pharmaceutical residues, PAHs, and PCBs, among others) existing in ecological samples are gaining popularity (Patel et al. 2019). When a transducer detects specific pollutants, the reporter proteins acting as microorganism produces a color signal (Zhang and Liu 2016). A biosensor designed for recognition and bioremediation should have increased microbe-contaminant interaction (Dhar et al. 2019). This allows bacteria to modify their biological processes in reaction to external environmental circumstances and codes the genes needed to use resistive substances as a substrate (Skinder et al. 2020). Since genetic circuits can be built in contrast to an exogenous environmental toxin, the strategies of synthetic biology are conceivable for detoxifying a specific harmful chemical. Two-component regulatory system (TCRS) synthetic genetic circuits were also developed for the bacteria (Ulu Seker et al. 2017). In a bacterial TCRS, histidine kinase (HK) and response regulator are present (RR). Here HK is a sensor domain protein with an extracellular loop that is a part of the membrane. HK features a transmitter domain, a highly conserved domain, in the last cytoplasmic transmembrane. As a result, using TCRS-based synthetic biology to construct biosensors for cell-mediated recognition and bioremediation could be a significant step forward.

6 PAH-degradation by Genetically engineered microorganisms (GEMs)

Although bioremediation for PAH-degradation has recently gained prominence as a field of study, it can occasionally be quite slow because of several biotic and abiotic variables. In lab tests, microbial species have the best capability to degrade PAHs, but in field trials, they do not perform well. Even though some microbial species may utilize PAHs as a source of carbon, improving their catabolic efficiency is necessary for total PAH degradation. The enzymatic activity of these microbial species could be improved to increase their capacity for PAH-biodegradation by genetic engineering. Therefore, the use of genetically modified or engineered microbes has the potential to degrade numerous types of pollutants, including PAHs. The breakdown of PAHs by GEMs with unique metabolic activity has received substantial attention. By modifying or manipulating the genetic traits of microorganisms, GEMs are created utilizing genetic engineering (Filonov et al. 2005). Various genetic engineering approaches, like DNA recombinant technology, genetic transposition, gene duplication, etc., have been created for the construction of new microorganisms for the removal of environmental toxins (Figure 2). To achieve high catalytic activity under environmental stress, these strategies induce controlled gene expression. Occasionally, local microbial species cannot survive in polluted environments. In comparison to laboratory size experiments, the degrading efficiency of such microorganisms may be lower in contaminated natural ecosystems. In cases when native microbial species are unable to digest PAHs, GEMs may be utilized as a substitute.

The use of GEMs in the bioremediation of PAHs has entered a new phase of the study. Numerous studies have demonstrated the tremendous potential of engineered microbes as PAH-degrading agents. Numerous aliphatic, aromatic, and polyaromatic hydrocarbons can be broken down by genetically modified multi-plasmid microbial strains (PAHs). This multi-plasmid microbial strain simultaneously oxidizes hydrocarbons while plasmid-specified breakdown pathways can be triggered in the presence of the right inducer. It was observed that this multi-plasmid bacterium grows on crude oil more quickly than microorganisms with a single plasmid (Sakshi et al. 2020). *P. putida* strain KT2442 has been altered using plasmid pNF142::TnModOTc. This recombinant strain of *P. fuorescens* HK44 with naphthalene degrading capabilities was the first GEM that was used for field application in the USA (Sayler and Ripp 2000). According to Filonov et al. (2005), the genetically altered *P. putida* KT2442 (pNF142:: TnMod-OTc) is more stable and exhibits more specific growth on naphthalene. The bioremediation activity of *Pseudomonas* sp can be improved through genetic engineering employing various molecular approaches as per several studies (Zhao et al. 2011). The use of GEMs could potentially improve pollutant dissolution. The GEM *P. putida* KT2440-rhlABRI was created by cloning the rhlABRI cassette containing the rhamnolipid-producing genes from *P. aeruginosa* strain BSFD5 into a random transposon vector. The rhlABRI cassette could be strongly expressed by this GEM, producing rhamnolipid. It was discovered that *P. putida* KT2440-rhlABRI can promote pyrene

Figure 2 The building fundamentals of synthetic biology used for bioremediation studies

breakdown by native microbial species in natural soil. As a result, *P. putida* KT2440-rhlABRI may increase the remediation of soils contaminated with PAHs (Cao et al. 2012).

For effective PAH-degradation increased catabolic enzymatic activity in GEMs. The GEM is made from *Pseudomonas* sp. CGMCC2953, a bacterium isolated from an oil-contaminated soil, demonstrated improved phenanthrene degradation capabilities. The PAH degradation gene C23O (catechol 2,3-dioxygenase) is present in this developed GEM. Effective cloning of the biodegradative C23O gene into plasmid pK4 was created from plasmid pRK415. Given that C23O is the primary enzyme involved in the breakdown of phenanthrene, *Pseudomonas* sp. CGMCC2953-pK was shown to be extremely effective. To effectively remove PAH (phenanthrene), it is, therefore, effective to increase the activity of important enzymes such as C23O (Wei et al. 2013). Using a gene encoded by catechol 2,3, -dioxygenase (C23O), the genetically modified dioxygenase-producing bacteria *P. putida* was created (nahH). The recombinant vector pUC18-nahH was effectively transferred into *P. putida* after this gene was cloned into the plasmid pUC18. When compared to biodegradation by the natural type, a significant improvement in PAH (phenanthrene and pyrene) biodegradation by genetically modified *P. putida* was seen (Mardani et al. 2017). A PAH-degrading microbial consortium built with two genetically modified fungal strains of *Aspergillus niger* exhibited significant levels of tolerance and high effectiveness of LMW and HMW PAHs degradation. These GEMs express the fungus *Phanerochaete chrysosporium's* LiP (lignin peroxidase, LiP+5 strain) and MnP (manganese peroxidase, MnP+7 strain) genes. These GEM strains demonstrated increased HMW-PAHs degradation and longer life in PAH-polluted soil (Zafra et al. 2017). Nevertheless, various studies have demonstrated that genetic engineering can be useful for pollutant biodegradation and offers a substantial opportunity for utilizing native microorganism capabilities for the creation of GEMs that are well adapted to polluted environments and more effective for

PAH-degradation. Unfortunately, these GEMs are only useful in favorable laboratory settings, and their potential for use in PAH-bioremediation at the field scale is constrained by many obstacles, including obtaining permission to release GEMs into contaminated environments and determining their fate as well as their monitoring, control, and risk to ecosystems and human health. Therefore, the key to achieving comprehensive and secure pollutant removal is genetic modification of the appropriate microbial species with rapid growth and effective biodegradation skills without any damage to the environment. To increase the reclamation of contaminated environments, further study is required into bioremediation using various microbes as well as GEMs.

Concluding Remarks

The most serious environmental issues in the world are hydrocarbon contaminants in the ecosystem. The investigation revealed that there has been an overuse of hydrocarbon pollutants, posing a severe environmental concern. Petroleum hydrocarbons rank among the toxins that are most dangerous to human and environmental health. Bioremediation utilizing microorganisms that degrade petroleum hydrocarbons is largely recognized as an ecologically beneficial and effective approach. A considerable number of bacterial species having the ability to degrade petroleum hydrocarbons have been identified and utilized in bioremediation. It is impossible to overstate the importance of developing strains that could be effective in the bioremediation of hydrocarbon-polluted locations. But it has been found that many problems slow down the effects of biodegradation. PAH removal from the environment is a difficult task. As a result, a thorough understanding of the mechanisms underlying the various remediation processes is critical. Enzymes, in contrast, play a critical function in biodegradation. Investigations of enzyme-substrate interactions and alterations are useful in guiding related experiments. Because of its convenience and inexpensive cost, molecular docking was extensively used in numerous research-based domains. Molecular docking, in particular, is capable of predicting and accounting for the procedure of biological reactions. Molecular docking can be utilized as a pre-experiment for investigating the characteristics of these contaminants and providing theoretical data for further research. The remediation techniques for the detoxification of xenobiotics and associated substances in the environment are also covered by synthetic biology.

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Conflicts of Interest

The authors declare no conflict of interest.

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