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Recent Application of Enzymes and Microbes in Bioremediation

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Abstract

Chemicals used in industry and military, along with poor waste management, cause soil, water, and air pollution. Pollutants pose health risks due to their resistance to degradation processes. Conventional methods are costly and generate secondary pollution. Bioremediation offers eco-friendly alternatives using enzymes and nanotechnology for efficient pollutant removal either in situ or ex sit. Microorganisms play a crucial role in bioremediation by converting toxic elements into less harmful compounds through processes like mineralization. They can survive in diverse environments and utilize various substrates, making them efficient in removing pollutants. Microbes utilize mechanisms like immobilization and mobilization to remove pollutants from the environment, with different types of bacteria specializing in degrading specific pollutants. Enzyme engineering involves manipulating biomolecules and processes for biotechnological applications. Two main strategies are rational design, requiring prior knowledge, and directed evolution, mimicking natural selection in a controlled manner. Rational design combines microorganisms or enzymes for specific reactions, while directed evolution creates gene variants through random mutagenesis for desired characteristics. Both methods aim to improve enzymes for bioremediation applications.

Keywords: Microorganisms, Microbes, Enzymes, Bioremediation, Pollutant

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Introduction

The extensive use of chemicals in all sectors of industry and military, insufficient waste management, and unintentional release result in the pollution of soil, water, and air. As an example, there are three hundred thousand polluted sites in Europe that require remediation. These contaminants pose a significant risk to humans, other organisms, and even the biogeochemical cycle. The durability, limited solubility, and resistance to several physical, chemical, and biological degradation processes of pollutants are the primary factors contributing to their toxicity (Padhye *et al.*, 2023). Various physical and chemical techniques have been employed to remove and purify contaminants, including oxidizing agents, electrochemical treatments, adsorption, ion exchange, and membrane filtration. Despite the effectiveness of conventional techniques in dealing with high concentrations of pollutants, they proved insufficient in reducing the level of contamination to regulatory thresholds (Kathi and Mahmoud, 2024). Constraints of conventional pollutant removal techniques include expensive nature, lack of specificity, and potential generation of secondary pollution (Razzak *et al.*, 2022). Consequently, environmentally friendly and biological approaches, known as bioremediation, have attracted attention.

Bioremediation refers to cost-effective and practical methods and technologies applied to reduce pollutants at their source and mitigate risks to the environment and human health (Mousavi *et al.*, 2021). The parameters of pollutants (such as chemical structure, hydrophobicity, and polarity), environmental conditions (including temperature, pH, and redox state), and soil characteristics (such as aggregation, thickness, dissolved organic matter, and pollutants aging) impact the process of biological degradation and the availability of contaminants (Ali *et al.*, 2023). Enzymes are highly effective bioremediation agents that facilitate all chemical transformations in contaminants. Moreover, it is feasible to modify the enzymes to improve their stability and effectiveness for specific circumstances or substrates. Nanoscale technologies play a crucial part in these advancements. Enzymes in bioremediation can be utilized either individually, with the separated enzyme being added to the contaminated region, or as entire cells obtained from bacteria, fungi, or algae (Mousavi *et al.*, 2021).

The utilization of individual enzymes in bioremediation offers several benefits over microbial whole cells. These advantages include enhanced specificity, easier manipulation and storage, standardized activity, increased mobility due to smaller size, activity in the

presence of high concentrations of toxic substances, and biodegradability that diminishes persistence and resistance (Bala *et al.*, 2022). The efficiency of this method is significantly higher for extracellular enzymes and cofactor-independent enzymes. The natural environment exhibits limited enzyme production, while it is feasible to enhance the synthesis of enzymes under regulated circumstances. Therefore, recombinant DNA technology and gene engineering offer numerous possibilities for the production of enzymes that are both more efficient and more abundant. Additionally, nanotechnology provides several methods to enhance the stability of enzymes by reducing their susceptibility to mechanical stress, maintaining the triatomic structure of enzymes, and safeguarding them against proteases (Anboo *et al.*, 2022). Enzymatic bioremediation can occur either via in situ or ex situ methods. In in situ techniques that cause minimal environmental disruption, the soil is supplemented with either free or immobilized enzymes (adsorbed enzymes on mineral supports that reduce the loss of enzymatic activeness). This mode of operation is more cost-effective as it eliminates the requirement for excavation and transportation of dirt. Ex situ techniques are viable for soils suffering from severe contamination with hazardous substances or when prompt intervention is necessary. This process involved excavating and treating soil in several bioreactors under optimal conditions for enzyme activity. Effluent enzymes such as mono- or dioxygenases, halogenases, peroxidases, phosphotriesterases, hydrolases, transferases, and oxidoreductases derived from diverse bacterial, fungal, algal, and plant species have been employed for the purpose of bioremediating contaminants (Mousavi *et al.*, 2021). This review provides a comprehensive analysis of the key enzymes involved in the bioremediation of contaminants and elucidate their specific mechanisms of action and modifications.

Microorganism in Bioremediation

Microorganisms can convert toxic elements into water, carbon dioxide, and other less toxic compounds, which are further degraded by other microbes in a process referred to as mineralization (Mahmood *et al.*, 2021). Microbes are ubiquitous in nature, and they utilize a wide range of substrates as carbon source; hence, they are found in unusual environments where they can absorb a wide range of pollutants (Kour *et al.*, 2022). Also, their ability to survive in odd environments promotes their efficiency. For example the acidophiles survive

in acidic environments, the psychrophiles thrive in cold climates and the halophiles survive in saline region (Kochhar *et al.*, 2022). Microbes can remove pollutants from the environment using different mechanisms. These mechanisms can be placed into two broad categories namely immobilization and mobilization. Mobilization process involves enzymatic oxidation, bioleaching, biostimulation, bioaugmentation and enzymatic reduction procedure. On the other hand, immobilization includes bioaccumulation, complexation, biosorption, and precipitation (solidification) (Ayangbenro and Babalola, 2018).

During mineralization, microbes help transform pollutants into end products such as carbon dioxide and water or other intermediate metabolic substances. Similarly, immobilization is the conversion of compounds into a form where it will be unavailable in the environment, for instance, the conversion of nitrate nitrogen into organic nitrogen (Dong *et al.*, 2022). The method is usually utilized for the bioremediation of heavy metals, especially in highly contaminated environments. Immobilization can be carried out using the in-situ and the ex situ methods. The ex-situ process involves the removal of polluted soils from the site of pollution to another location where it would undergo a microbial process to immobilize the metal ions responsible for the contamination (Ayangbenro and Babalola, 2018). On the other hand, in the in-situ procedure, the pollution is treated on site. Microbes such as *E. asburiae* and *B. cereus* have been reported to be involved in immobilization of heavy metals which pollute the environment (Wróbel *et al.,* 2023). During microbial bioremediation, microbes protect themselves from toxic compounds by forming hydrophobic or solvent efflux pump that protects the outer membrane of the cell (Ayilara and Babalola, 2023). Aerobic bacterial species such as Mycobacterium, *Alcaligens, Sphingomonas,* and *Pseudomonas* are known for their aerobic degradation of hydrocarbons (alkanes and polycyclic aromatic hydrocarbons) and pesticides (Elyamine *et al.*, 2021). Along with that, a few of the aerobic methylotrophs are also recognized for the degradation of dichloroethane and trichloroethylene (chlorinated aliphatics). Some of the anaerobic bacteria species are known for the degradation of polychlorinated biphenyls (PCBs), chloroform, and trichloroethylene (chlorinated solvent).

Enzyme Engineering

Biomolecular engineering is a relatively new field of academic research and industrial practice with a goal of engineering biomolecules, such as proteins and nucleic acids, and biomolecular processes for biotechnological applications (Nagamune, 2017). In recent years, two different, yet complementary strategies in biomolecular engineering have been developed to genetically engineer enzymes or microorganisms for bioremediation applications: rational design and directed evolution (Figure 1). The rational design approach for bioremediation typically involves either the construction of a single microorganism in which desirable biodegradation pathways or enzymes from different organisms are brought together to perform specific reactions using recombinant DNA technology (whole cell level), or the engineering of enzymes with desired characteristics using site-directed mutagenesis (protein level) (Rafeeq *et al.*, 2023). Enzymes are delicately folded proteins, where even small changes in the amino acid sequence can disrupt the protein configuration and diminish catalytic activities. Moreover, it is near impossible to predict the impact of a modification in a single trait of the enzyme on other biochemical properties. Thus, to successfully modify an enzyme using rational design, a huge amount of a priori information on the structural, mechanistic and dynamic properties of the protein is required (Ndochinwa *et al.*, 2024). This places an enormous demand on manpower and laboratory resources. Nonetheless, the capability of rational design is rapidly improving due to recent advances in enabling technologies such as X-ray crystallography and bioinformatics.

In contrast to rational design, directed evolution does not require a priori knowledge of the protein structure and can identify mutations that influence enzyme activity through subtle long-range interactions. This approach mimics a simple algorithm that nature has successfully used over eons of time, i.e. genetic diversification coupled with natural selection pressure (Ang *et a*l., 2005). However, unlike natural evolution, directed evolution has a specific goal that is empirically controlled in the laboratory, and can collapse the process into a matter of months or even weeks. In essence, directed evolution involves the creation of a diverse library of gene variants through random mutagenesis, such as error prone PCR or gene recombination techniques, such as the in vitro staggered extension process (StEP) recombination (Lane and Seelig, 2014) and in vivo DNA shuffling, followed by selection or screening, to obtain the enzymes or pathways with the desired characteristics (Ndochinwa *et al.*, 2024). The process is iterative where the selected or screened enzymes are subjected to further rounds of random mutagenesis or gene

recombination to produce a new generation of enzyme or biochemical pathway variants in a microorganism. For the bioremediation of POPs, this means the generation of a genetically capable organism or enzyme for the complete biodegradation of the compound of interest

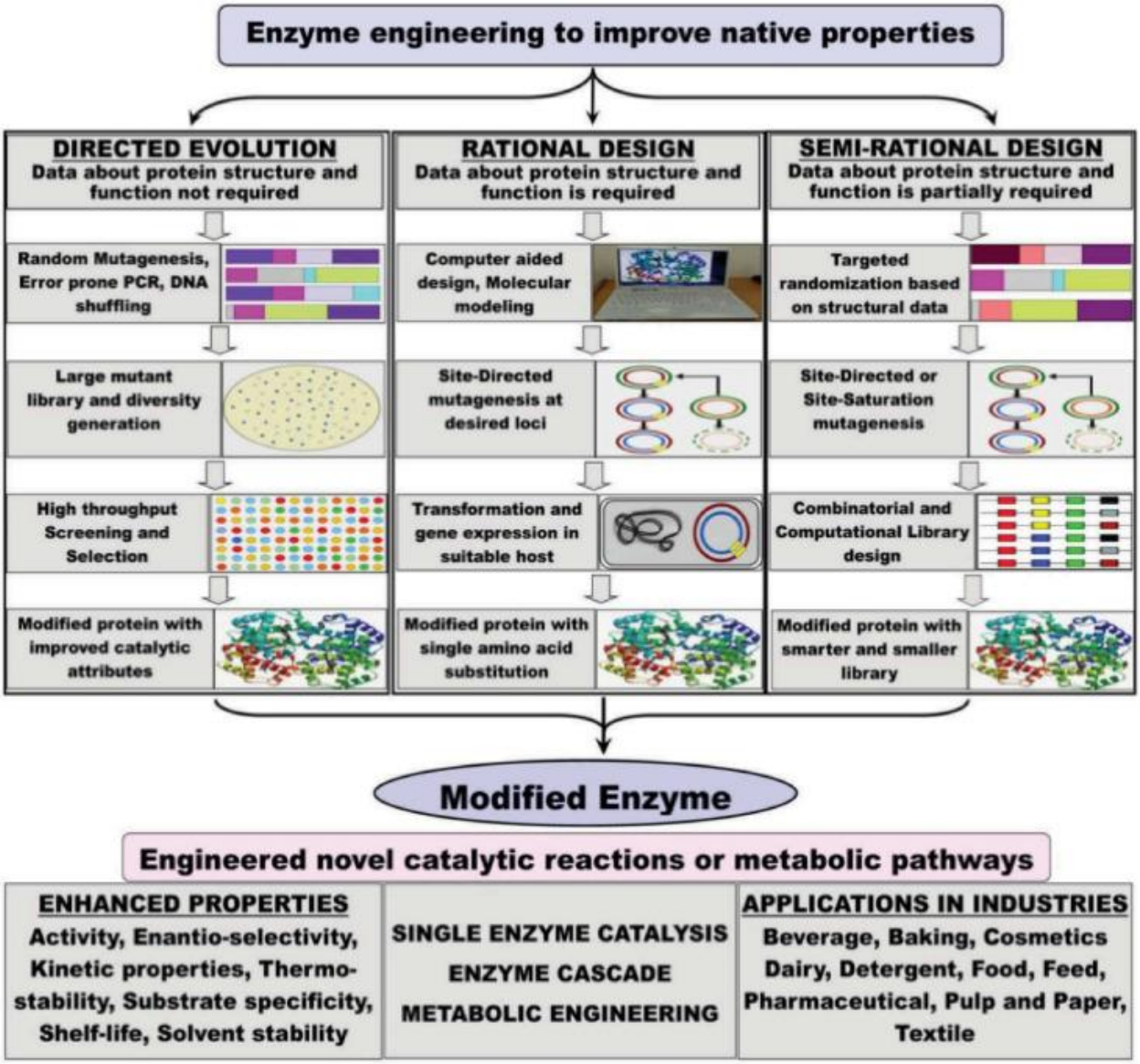


Figure 1: Major enzyme engineering strategies (Adopted from Ndochinwa *et al.*, 2024).

Algae as Bioremediation Agent

Algae bioremediation, also known as phycoremediation is a unique technology because it is a self-sustaining cycle. To oxidize contaminants into less-harmful metabolites, algae extract and utilize oxygen from its surrounding environment; these metabolites include CO_2 and

H_2O. For growth, algae use photosynthesis, which requires CO_2 and H_2O (Daneshvar *et al.*, 2022). Photosynthesis, in turn, releases oxygen that algae can employ for further contaminant oxidation, thus repeating the cycle. Oil spills devastate ecosystems and the atmosphere by tainting water sources, releasing toxic vapors, and causing irreversible contamination to aquatic and terrestrial habitats (Mohsenpour *et al.*, , 2021). For ecosystems, crude oil contamination directly kills producers and consumers within the proximity of the spill.

Algae form an important component of the microbial community that inhabits the aquatic and terrestrial environments. Different species have been observed growing spontaneously on the surfaces of water, at the shorelines or at the bottom of freshwater bodies where they find nutrients (Cruces *et al.,* 2010). Many of them have also been found growing and thriving unaided in sewages and even in severe crude oil-polluted terrestrial and aquatic environments; a clear indication that they can utilize crude oil as nourishment (Aditi and Suneetha, 2015). Macroalgae have been used for a long time in the removal of pollutants in the environments, mostly heavy metals and inorganic industrial pollutants (Abioye *et al.*, 2020). These treatments have mostly been applied on soil environments or at wastewater discharge site (Nithiya *et al.*, 2018). Only very few accounts of their involvement in the biodegradation of hydrocarbon are available; with Chlorella, Scenedesmus, and Raphidocelis species tested on PAHs degradation (Aditi *et al.*, 2015; Nithiya *et al.*, 2018). This is partly because more studies have not been conducted on the hydrocarbon-degradation potentials of algae and their metabolic pathways on the various crude oil components. Hence, information about algal species that strictly utilize hydrocarbon as sole carbon source is very scanty (Han *et al.*, 2019). However, with the expansion of interest on how to manage hydrocarbon contaminants, there is a strong belief that the application of algae in the cleaning of petroleum-polluted environments may just be the missing piece of the puzzle.

Remediation of Wastewater

Wastewater runoff from various pointed and nonpointed sources including household, agricultural practices, and industrial sectors contain carbon-rich residues along with harmful pollutants that contaminate the environment and cause undesirable changes in the

water ecosystem which results in disastrous effects on human health and other living in the environment (Karimi-Maleh *et al*., 2020).

Wastewater is sometimes not treated before being discharged into waterways which causes significant problems, particularly where a microbial organism, biochemical oxygen demand, toxicity, and nutrients are found in combination (Siddiki *et al*., 2021), wastewater can be used as a nutrient medium in waste biorefineries for the cultivation of valuable organic biomass, namely microalgal biomass (Bhatia *et al*., 2021). *Chlorella sorokiniana* has been tested for the treatment of raw municipal wastewater achieving partial decrease of major pollutants, as well as for the removal of organic micro-pollutants from water and wastewater (Kotoula *et al*., 2020). A 7 days culture of *Chlorella sorokiniana* was prepared using microbiological method and used to treat wastewater samples by Badamasi *et al*. (2024). The medium was incubated under the temperature of 25°C and provided with 12/12h light/dark photoperiods for four weeks.

The result from the research shows that phycoremediation with *Chlorella sorokiniana* leads to the accumulation of biomass rich in biochemical components, including lipids, carbohydrates, and proteins. These results underscore the potential of *Chlorella sorokiniana* as a promising candidate for sustainable wastewater treatment strategies. The study affirms the remarkable reduction capacity of *Chlorella sorokiniana* particularly in total dissolved solids, phosphate, ammonium, nitrate, potassium, and heavy metals. The study recommends more research, focused on optimizing the cultivation techniques of *Chlorella sorokiniana* to enhance its growth and wastewater remediation efficiency.

Remediation of Dye

Dyes are natural or synthetic coloured substances that are used to impart colour to various substrates (Kumar *et al*., 2021). They are of various types based on their dissociation in aqueous solutions and can be water-soluble or insoluble depending on their mode of application. The complex aromatic structure and high stability of dyes makes them very difficult to remediate, thus, their indiscriminate release into the environment may lead to a potent hazard to natural sources like soil, water, flora, fauna, livestock and human population (Jamee and Siddique, 2019). The most common dye-remediation strategy is the microbial remediation which involves the use of microorganisms from different species, which proves to be very effective. Microorganisms are ideally suited to the task of remediating dyes due to their size, adsorption capacity and possession of enzymes that

allow them to utilize the dyes as food. Microorganisms have served in nature for billions of years in the breakdown of complex human, animal, and plant wastes maintaining the continuity of life from generation to generation (Tripathi *et al.*, 2023).

Bacterial remediation of synthetic dyes involves a high degree of removal and mineralization of harmful compounds which is economically viable, results in minimal production of sludge and is faster than that performed by fungi (Panwar *et al.*, 2023). Bacteria mineralize dye by enzymatic action and/or biosorption (Tripathi *et al.*, 2023).

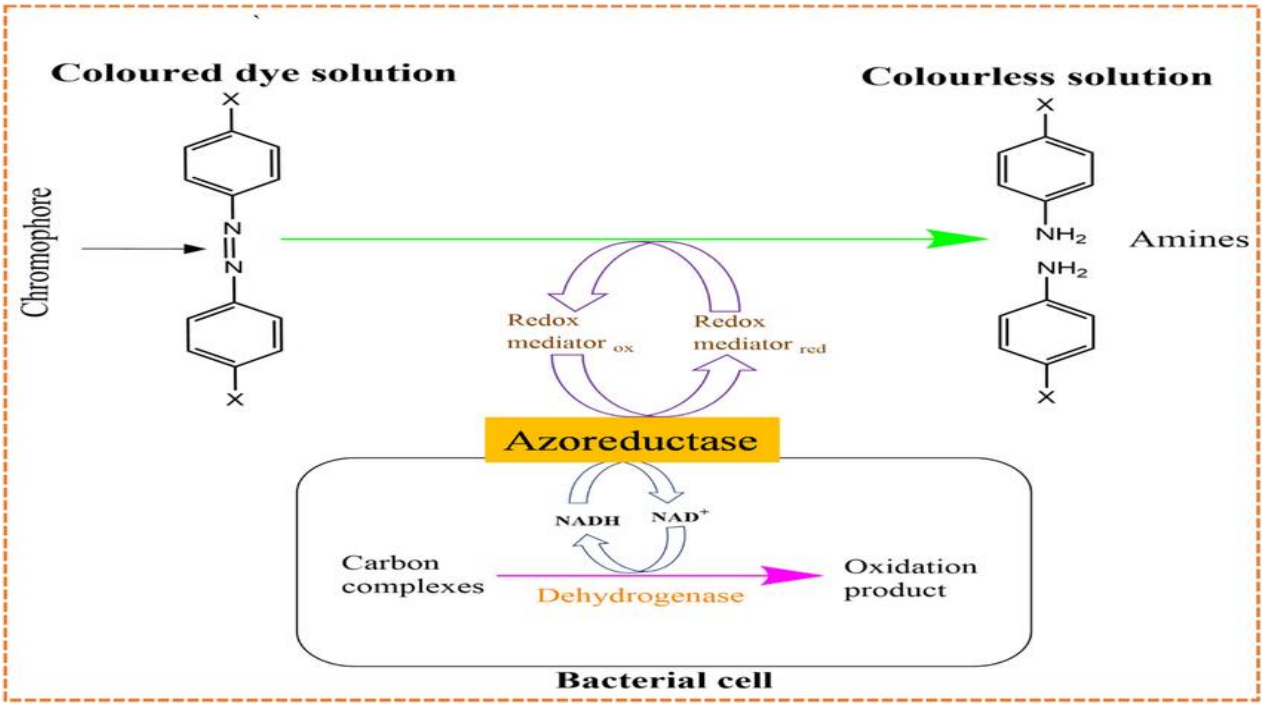


Figure 2: Mechanism of remediation of dye-contamination by bacteria (Tripathi *et al.*, 2023).

Bacterial enzymatic remediation of dyes involves breaking of the azo bonds (reductive cleavage) by azoreductase and dissociation of other functional groups in the dye molecule (as shown in figure 2) by enzymes like peroxidase, laccase, tyrosinase, NADH-DCIP reductase and monooxygenase reductase (Huy *et al.,* 2020). Some of the azo dyes that can be detoxified and decolourized by bacteria include amaranth, naphthalene, acidic and anthraquinone (Shi *et al.*, 2021). Enzymatic remediation by bacteria occur aerobically, anaerobically, or through both (Mishra *et al.*, 2022). The remediation of dyes under aerobic conditions is not very efficient due to the oxidative stability of most of the dyes resulting to their inability to completely adsorp on activated sludge (Rima *et al.*, 2022). Aerobic bacteria

have been reported to metabolize sulfonated azo dyes by producing enzymes that cleave the azo bonds (Franciscon *et al*., 2012). Sari and Simarani (2019) isolated reductases from *Pseudomonas sp*. KF46 which were used in the aerobic degradation of some azo dyes. *Klebsiella sp*. and *Staphylococcus sp*. also decolourized 90 % of a dye under aerobic condition (Desai, 2017). The aerobic degradation of acid red 151 resulted in the production of aromatic amines and some carcinogenic metabolites (Prasad and Aikat, 2014). In a study involving bacterial aerobic degradation of Para red and Sudan I dye, an oxidative burst occurred due to the formation of some metabolites (Mishra *et al*., 2022). Most aerobic bacteria require a longer period of acclimatization in the presence of azo compounds to induce the expression of azoreductase, thus, show high specificity to most of the dyes (Cui *et al*., 2014). Remediation of dyes by bacteria also occur anaerobically through redox reaction of hydrogen to form methane, carbon dioxide, hydrogen sulfide and other gaseous compounds, subsequently releasing electrons (Ayele *et al*., 2021). This process involves direct transfer of electrons to the dyes as terminal acceptors in the presence of enzymes and reduction of the dyes through bacterial catabolism. This method of remediation is more efficient and economical than aerobic method, as it is mostly specific and occurs under static conditions (Thangaraj *et al*., 2021). Reactive red 120 was degraded anaerobically by an unidentified bacterial species (Mishra *et al*., 2022). In some cases, both aerobic and anaerobic bacteria remediation is employed to completely break down of dyes. Synthetic dyes like disperse blue 79, fast acid red GR and dark red 2B were successfully degraded through the anaerobic-aerobic method (Desai, 2017).

Bacteria serve as good biosorbents of dyes, as they require carbon and nitrogen which are main constituents of the dyes. Bacterial biosorption is mainly used for the mineralization of nondegradable pollutants from effluents, such as metal ions and dyes. It is one of the efficient ways of remediating pollutants (Mustapha and Halimoon, 2015). During biosorption, electrically charged functional groups on bacterial cell surface interact with the dye molecule to form a strong bond, subsequently dissociating to form less harmful compounds (Srinivasan and Viraraghavan, 2010; Tripathi *et al*., 2023). Several Bacillus species (*Bacillus subtilis*, *B. cereus*, *B. megaterium*, *B. fusiformis, B. odysseyi, B. mycoides, B. paramycoides, B. pseudomycoides, B. flexus, B. cohnii, B. licheniformis, B. spizizenii, B. algicola, B. vallismortis, B. vietnamensis, B. stratosphericus, B. halodurans, B. albus, B. aryabhattai* and *B. velezensis*) have shown to be very effective in the remediation of different varieties of synthetic dyes either as individual pure cultures or in mixed culture with other

microorganisms. This has help in the suppression of the impact of these dyes to the environment. However, the introduction of new biotechnological techniques has resulted in the continuous discovery of new species of Bacilli that have the capability to remediate dyes and other environmental contaminants, thus, more species may be reported in the near future.

Remediation of Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons are aromatic compounds made up of two or more fused benzene rings. PAHs are recalcitrant and can persist in the environment for long periods, but are conducive to biodegradation by certain enzymes found in bacteria and fungi (Patel *et al.*, 2020). In the past several years, several oxidoreductases such as laccases and cytochrome P450 monooxygenases (CYPs) have been exploited for the enzymatic degradation of PAHs. Laccases belong to a group of multicopper enzymes that can catalyze the oxidation of a wide variety of phenolic compounds including PAHs. Like other ligninolytic enzymes, laccase is difficult to express in non-fungal systems and knowledge of structure–function relations underlying the key functional properties of laccase is limited. Hence, directed evolution holds exciting potential for improving the performance of the enzyme. In a study undertaken by Viswanath *et al.* (2014), the laccase gene from Myceliophthora thermophia (MtL), which was previously expressed only in Asperigillus oryzae, was transformed into Saccharomyces cerevisiae and subjected to directed evolution. After 10 rounds of directed evolution, a mutant with a 8-fold increase in laccase expression and a 22-fold increase in the kcat for 2,2-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) was created. The final mutant had a total activity 170-fold higher than the wild type. The MtL enzyme holds great potential for the bioremediation of PAHs due to its high thermal stability that enables it to work at the elevated temperatures needed to increase the solubility of highly recalcitrant PAHs. However, this particular enzyme has yet to be tested on actual PAH degradation. It is noteworthy that, although laccases are promising catalysts with a variety of potential uses, their applications have been limited by their requirement for mediators. Thus, engineering of a laccase with activity in the absence of mediators would be a suitable target for directed evolution.

CYPs are one of the largest known enzyme superfamilies and are expressed in most living organisms. PAHs can be oxidized by CYP enzymes to form catechols, which are then

degraded by other enzymes, including catechol dioxygenases to harmless products and incorporated into the tricarboxylic acid cycle of microorganisms (Stoddard *et al.*, 2021). Wild-type CYP101 (P450cam) from Pseudomonas putida has been shown to have an inherently low activity towards the PAH substrates phenanthrene, fluoranthene, pyrene and benzo[a]pyrene (Ang *et al.*, 2005). Therefore, CYP enzymes have been subjected to a number of rational design studies to enhance their catalytic performance. Based on the crystal structures of CYP101, selective mutations were performed on the active site residues F87 and Y96 of the enzyme (Harford-Cross et al., 2000). For all PAH substrates studied, the absolute oxidation rates (approximately 1 min−1) of the mutants, Y96A, Y96F, F87A/Y96A and F87L/Y96F, were increased by two to three orders of magnitude relative to the wild-type enzyme. In a similar study, based on the crystal structure of the enzyme, Carmichael and Wong (Carmichael and Wong, 2001) introduced two mutations into CYP102, R47L and Y51F, and found that the oxidation activity of the enzyme for phenanthrene and fluoranthene was increased by 40- and 10-fold, respectively.

Remediation of Polychlorinated Biphenyls

Polychlorinated biphenyls are a class of chemicals consisting of 209 member compounds, collectively known as congeners. PCBs can be degraded by microorganisms via a metacleavage pathway to yield tricarboxylic acid cycle intermediate and chlorobenzoate (CBA). The initial step in the aerobic biodegradation of PCBs is the dioxygenation of PCB congeners by the biphenyl dioxygenase enzyme. In this step, the enzyme catalyzes the incorporation of two hydroxyl groups into the aromatic ring of a PCB congener, which increases the reactivity of the PCBs, rendering them more susceptible to enzymatic ring fission reactions (Steliga *et al.*, 2020). Biphenyl dioxygenase is a multicomponent enzyme consisting of a terminal dioxygenase (made up of a large and a small subunit), ferredoxin and ferredoxin reductase encoded by the *bph* operon (Furukawa *et al.*, 2004). The substrate recognition of the enzyme is controlled by the large subunit encoded by the *bphA* gene.

By targeting a fragment of *bphA* gene that is critical for enzyme specificity and using DNA shuffling techniques to shuffle particular gene fragments from B. cepacia strain LB400, C. testosteroni B-365 and Rhodococcus globerulus P6, Barriault *et al.* (2002) were able to obtain variants with superior degradation capabilities for PCBs. The hybrid BphA, II-9, was able to oxygenate 2,6-dichlorobiphenyl, which is a very persistent PCB congener, by up to 58% after 18 hours.

Remediation of Organophosphates

Organophosphates (OP) are highly toxic neurotoxins used in insecticides and chemical warfare agents. Included in the organophosphate group are paraoxon, parathion, chlorpyrifos disulfoton, ruelene, carbophenothion and dimeton. The neurotoxicological properties of this class of compounds are mainly due to its ability to suppress acetylcholinesterase and as a result, prevent acetylcholinesterase from breaking down acetylcholine at the synaptic junction. These compounds have also been associated with pathology and chromosomal damage associated with bladder cancer (Firozjaei *et al*., 2015). Bacterial phosphotriesterase (PTE), also known as organophosphorus hydrolase (OPH) is a highly efficient hydrolytic enzyme that can hydrolyze a broad range of organophosphates (Ambreen *et al*., 2020). PTE catalyzes the cleavage of P–O, P–F or P–S bonds in these organophosphates. With paraoxon, its preferred substrate, it has a kcat of about 2280 s^{-1} and a kcat/KM of 6.2 × 107 $M^{-1}s^{-1}$, which are very close to the maximum diffusion-controlled limit (Hong and Raushel, 1999). As such, application of PTE to the degradation of organophosphates has attracted considerable research interest. Although PTE can hydrolyze a variety of organophosphates, it generally prefers the Sp-enantiomers of organophosphate over the Rp-enantiomers, with the kinetic constants of the Sp-enantiomers being higher by one to two orders of magnitude (Katyal *et al*., 2020). To make PTE more effective for the catalytic degradation of organophosphates, the overall rate of hydrolysis for all stereoisomers must be increased. The three-dimensional structure of PTE from Pseudomonas diminuta with a bound substrate analogue, diethyl 4-methylbenzylphosphonate, has shown that there are three distinct hydrophobic binding pockets responsible for the orientation of substrates in the enzyme's active site (Zhang *et al*., 2009; Dym *et al*., 2023). These three binding pockets have been named the small, large and leaving group subsites.

To determine the role played by each subsite and the key amino acid residues in the reactivity and stereospecificity of PTE, Chen-Goodspeed *et al.* (2001) carried out site-directed mutagenesis on key residues in the three subsites and measured the stereospecificity of the variants using a series of asymmetric organophosphates. The substitution of Ile106 by a smaller alanine residue resulted in an enlargement of the small subsite and virtually eliminated a 20–90-fold preference for Sp-enantiomers of some chiral substrates. A combined mutation of I106G/F132G, which also enlarged the small subsite, brought about a kcat increase of up to 270- fold for some of the Rp-enantiomers without

sacrificing the high turnover rates for the Sp-enantiomers, which is a highly desirable property for the remediation of a racemic mixture of organophosphates. When the His257 residue in the large subsite was mutated to a tyrosin residue, which reduced the size of the large subsite, the kinetic parameters of PTE on all tested Sp-enantiomers were reduced. This indicates that His257 plays an important role in the stereoselectivity of PTE.

Table 1: Microbes Utilized in Bioremediation of Some Heavy Metals

Heavy metal	Toxicity	Microbe
Arsenic	The inorganic forms (As^{3+} (arsenite) and As^{5+} (arsenate)) are toxic and may cause enzyme inactivation, carcinoma, hemolysis, keratosis, gangrene, and neurological and cardiovascular diseases	*Acinetobacter* sp., *Pseudomonas* sp., and *Sporosarcina ginsengisoli*, *E. coli, Bacillus idriensis*, *Sphingomonas desiccabilis* , *Rhizobium* sp., *Rhizopus* sp., *Trichoderma* sp., *Aspergillus flavus*, and *Penicillium canescens*, *Saccharomyces cerevisiae*, *Hydrodictyon, Oedogonium, Rhizoclonium,* and even a plant, *Pteris vittata*
Lead	Lead toxicity may cause anemia and appetite loss and gastrointestinal, neurological, and reproductive disorders	*Agaricus bisporus, Rhizopus nigricans, Penicillium canescens, Penicillium chrysogenum, Saccharomyces cerevisiae, Aspergillus niger,* and *Aspergillus terreus*, *Arthrobacter* and *Phaeolus schweinitzii, Cupriavidus metallidurans* , *Staphylococcus epidermidis*
Mercury	Hg toxicity would cause neurotoxicity, nephrotoxicity, allergies, and inability to speak	*Rhizopus arrhizus, Penicillium canescens, Geobacter sulfurreducens, Pseudomonas putida, Acinetobacter calcoaceticus, Staphylococcus aureus, Shigella flexneri*, *Enterobacter, Pseudomonas*, *Bacillus* spp *Pseudomonas, Aeromonas, Staphylococcus, Escherichia, Citrobacter, Bacillus,* and *Rhodococcus, Shewanella oneidensis*
Chromium	Cr (VI) is the most toxic heavy metal because of its high oxidative potential causing cell damage and mutagenic, carcinogenic, and teratogenic effects	*Pseudomonas, Bacillus, Escherichia, Shewanella, Enterobacter*, and *Thermus*

Mousavi *et al.* (2021)

Conclusion

Human actions have caused extensive contamination of the environment. Several organic pollutants, including polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and pesticides, are hard to break down and continue to pose a toxicological risk to wildlife and humans. In recent years, an increasing amount of potential dangers associated with the widespread existence of POPs in the environment have been documented. Bioremediation is a more cost-effective option than traditional physico-chemical methods for treating POPs at contaminated sites, as it can selectively break down pollutants without harming the site or its native plants and animals. Nevertheless, while bioremediation technologies have been praised as a cure-all for addressing polluted environmental media, their use has been restricted so far because of issues such as inconsistent substrates and environmental conditions, as well as the limited ability of naturally occurring microorganisms to degrade pollutants effectively. Specifically, the engineering and environmental introduction of GEMs has faced technical and ethical challenges, resulting in significant limitations for their practical use in the field. With the recent advancements in biomolecular engineering, it is now possible to sidestep natural evolution and break down environmental xenobiotic pollutants. This has created new opportunities for improving bioremediation programs in the years to come.

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