

Organic Pollutants Remediation by Enzymes from Microorganisms

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Abstract: *In the current global setting, environmental pollution is a substantial contributor to severe environmental deterioration. The threat posed by numerous contaminants has seriously harmed the survival of people, animals, and plants. There is an urgent need to stop the problem of organic wastes being released into the environment on a daily basis from diverse companies. Different approaches to managing organic pollutants are insufficient since they exacerbate already existing issues. Because enzymes naturally catalyze the conversion of many of the problematic substances, even when the compounds are present in the water at low amounts for "chemical reaction," enzyme-mediated bioremediation is a viable method. The current review looked at using microorganism-produced enzymes to remove organic contaminants from the environment. This study will give a thorough overview of the most recent developments in the field of pollution treatment and degradation and will primarily concentrate on enzymes that break down pollutants.*

Keywords: Organic Pollutants, Microorganisms, Enzymes, Bioremediation

1. INTRODUCTION

The growth of enterprises across a variety of industries produces garbage that is hardly decomposable and contains dangerous organic chemicals. Without effective waste management systems, the continual discharge of these hazardous materials into the water, air, and soil inexorably degrades the quality of our ecosystem and poses a threat to human health. The pharmaceutical industry, petrochemical refineries, textile manufacturers, dye and chemical manufacturing, and others all produce pollutants (Liu *et al.*, 2020). A variety of hazardous substances, including Polycyclic Aromatic Hydrocarbons, have been the subject of numerous research (PAHs). The US Environmental Protection Agency (EPA) has discovered more than 400 different types of PAHs and their effects, 16 of which have been classified as priority contaminants (Mojiri *et al.*, 2019). Because they prefer attachment to organic matter, are insoluble in water, and have a preference for fatty acids, chemical compounds known as PAHs are both hydrophobic and lipophilic. Because of these traits, PAHs accumulate in soils and sediments, where they become less bioavailable and more challenging to breakdown (Kronenberg *et al.*, 2017). For example, phenanthrene is listed as one of the 16 PAHs that the USEPA considers to be priority pollutants due to its shown toxicity.

In effluent from petroleum refineries, it is frequently present at concentrations ranging from 7.6 g L⁻¹ to 9.9 g L⁻¹, although it is typically present in natural waterways at concentration levels around pg L⁻¹ and not surpassing ng L⁻¹ (Wang *et al.*, 2019). Similar to how the atmosphere is influenced, the European Commission's (EC) laws and regulations on air quality and waste air treatment are increasingly common in terms of managing and controlling industrial waste (Tandjaoui *et al.*, 2019). Significant amounts of volatile organic compounds (VOCs) are directly responsible for the greenhouse effect, stratospheric ozone depletion, and photochemical haze (Zhang *et al.*, 2017).

Global public health challenges are being created by the weight of illness and mortality linked to environmental toxins (Xu *et al.*, 2018). Over the last few decades, a wide variety of pollutants with various structures and ecotoxicities have been steadily polluting the environment. These anthropogenic pollutants, which are emitted from a variety of sources, pose a serious risk to the stability of ecosystems and to public health, causing immune system impairment, cancer, and type 2 diabetes (Claus *et al.*, 2016). Typically, organic and inorganic contaminants are separated out for environmental pollution.

Organic pollutants, such as pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), etc., are widely dispersed in the environment. Persistent organic pollutants (POPs), such as aldrin, DDT, chlordane, and hexachlorobenzene, are of special concern. Due to their inherent chemical stability, recalcitrance, acute toxicity, and mutagenicity or carcinogenicity, these POPs are exceedingly dangerous to human health. Modern industries synthesis a significant quantity of POPs that are discharged into the environment as a result of the acceleration of global urbanization.

Despite the danger posed by POPs, agricultural practices continue to be a major source of environmental pollution. Pesticides have been used excessively during the traditional agricultural process, which has resulted in ongoing environmental degradation. Due to the poor degradation of these contaminants by natural bacteria and plants, they remain in the environment for many years. They may also travel enormous distances by wind and ocean current, especially to the Polar Regions and open oceans. Pollutants' long-term

residual, bio-accumulative, and hazardous characteristics raise concerns about their presence in the environment. As a result, research into novel, efficient methods of pollutant degradation has gained attention on a global

The safest and greenest method for removing anthropogenic chemicals from ecosystems was thought to be bioremediation. It has become possible to identify and describe a large number of microorganisms and microbial enzymes with potential bioremediation capabilities (Wu *et al.*, 2017; Dvorak *et al.*, 2017). However, in other instances, they are either useless in the actual natural environment or are unable to totally decompose the intended pollutant. The utilization of these degradation enzymes has drawn a lot of interest, though, as our understanding of the catalytic mechanisms involved in the breakdown of contaminants has improved. Many enzymes that degrade contaminants have been used in industry and modern agriculture for things like biosensors and medicine production (Han *et al.*, 2017; Takano *et al.*, 2020).

Bioremediation refers to the remediation procedures made possible by the presence of microorganisms at the many polluted settings (Basu *et al.*, 2018; Kumar *et al.*, 2019). Multiple metabolic processes that produce enzymes are used in microbial remediation (Sharma *et al.*, 2018; Dangi *et al.*, 2019). These enzymes mostly participate in the processes through which xenobiotics are degraded (Junghare *et al.*, 2019). Depending on whether bioremediation is being done in-situ or ex-situ, there are many traditional approaches. To minimize soil disturbance, in situ is used on the site. This approach is primarily used since it saves money by not excavating or transporting polluted soil.

The primary in situ bioremediation techniques include bioaugmentation, bioventing, biosparging, and designed in situ bioremediation (Azubuiké *et al.*, 2016). Slurry phase system and solid phase system ex situ bioremediation techniques include composting, land farming, and biopiling (bioreactors). Solid and slurry phase systems are used to transport soil to speed up microbial degradation, while ex situ bioremediation is used to remediate home, industrial, and organic waste. Traditional bioremediation techniques require a lot of time and money yet produce inferior results.

Traditional bioremediation techniques demonstrated the aforementioned drawbacks of taking more time, removing or dissipating fewer pollutants, disrupting nature's delicate balance by covering more land for an extended period of time, and leaving the environment with an unpleasant odor (Bharagava *et al.*, 2019; Dangi *et al.*, 2019; Kumar, 2019). For the greatest outcomes, researchers are therefore eager to find innovative bioremediation strategies. The treatment of wastewater and organic pollutants by enzymes from microorganisms will be thoroughly outlined in this study's complete analysis of recent scientific developments.

2. PRESENT PROBLEMS AND ESSENCE OF BIOREMEDIATION

In the current global setting, environmental pollution is a key contributor to considerable environmental damage. By releasing numerous dangerous substances into the environment through effluents, the paper industry makes a significant contribution. Due to their resistive and slow degradation capabilities, phenolic and chlorinated pollutants seem to be the most hazardous and difficult (Azubuiké *et al.*, 2016).

The conversion of many hazardous substances can be selectively catalyzed by enzymes in their natural state, even when the compounds are present in the water at low concentrations for "chemical reaction," making enzyme-mediated bioremediation a promising method. As was already mentioned, lignin modifying enzymes are promising biocatalysts for long-term reduction of a variety of pollutants of concern because they have the capacity to environmentally friendly degrade a wide range of lignin, methoxylated compounds, phenol, polyphenol, EDCs, and non-phenolic compounds. Immobilization of the enzyme (Zdarta *et al.*, 2019) and the use of modified microorganisms (Dvoak *et al.*, 2017) can both increase the catalytic potential and yield of lignin-modifying enzymes.

2.1. Traditional methods of bioremediation for the reduction of pollutants

A promising technique called bioremediation uses plants and microorganisms from nature to remove hazardous organic pollutants in a "eco-friendly" manner (Tekere, 2019). The majority of modern bioremediation techniques center on biodegradation, which entails removing all harmless organic harmful compounds from a severely contaminated medium or site. It has been shown that a variety of biological degradation processes and pathways function both with and without oxygen (Ghattas *et al.*, 2017; Wang *et al.*, 2019). The goal of bioremediation research is to strengthen the remediation process by supplying the right amounts of biocatalysts, chemicals, and nutrients, typically including oxygen, that are required for the microbial metabolism and/or enzymatic conversion that breaks down and detoxifies toxic components.

In microbial assisted pollutant remediation, harmful substances are either entirely converted into water or carbon dioxide (organic pollutants) or are catalyzed into less dangerous forms (Malla *et al.*, 2018). This technology offers an effective substitute for traditional chemical treatment procedures because of its low cost and biology-based methodology. In comparison to physical and chemical methods for treating different environmental toxins, bioremediation is therefore seen as a cost-efficient, adaptable, efficient, and environmentally friendly alternative.

A few remediation methods rely on enzymatic systems produced from bacteria, while others use bioreactors and a few more methods use plant-based methods. In situ or ex situ pollutant mitigation is used to remove pollutants from a contaminated site using a bacterial-mediated remediation technique. According to the definition of in situ, bioremediation takes place on-site or within the contaminated area. Ex situ, on the other hand, denotes the possibility of applying microbiological clean up away from the contaminated site.

Remediation in situ is thought to be slow and usually challenging in the natural environment to regulate and optimize different bioremediation parameters. Use of specifically crafted bioreactors to speed up remediation is helpful in this situation and for ex situ

remediation. In order to provide the ideal conditions for aeration, microbial growth, and biodegradation to suit the various bioremediation goals, bioreactors have been designed for use in bioremediation procedures. According to reports, certain organic pollutants can be bioremediated using bioreactors such as packed, stirred tanks, airlift, slurry phase, and partitioning phase reactors (Pathak *et al.*, 2020).

Over the past few decades, a wide variety of bacterial species that produce lignin-modifying enzymes have been identified. These enzymes have been investigated for their ability to remove lignin, chlorinated lignin, organic phenol, and chemicals that disrupt human endocrine systems (Bilal *et al.*, 2019; Falade *et al.*, 2018; Grelska and Noszczyska, 2020). As a dye-decolorizing peroxidase, a more recent peroxidase enzyme has been identified. This enzyme has the potential to be employed as a biocatalyst to remove a variety of hazardous contaminants from wastewater. The chemicals that this enzyme affects include lignin derivatives, dyes, and EDC compounds (Brissos *et al.*, 2017).

2.1.1. Biostimulation

This type of bioremediation technique involves fostering local microorganisms on the site by adding the necessary nutrients (soil and groundwater). It typically focuses on enhancing the metabolism and metabolic pathway of the natural or indigenous microorganisms, whether bacterial or fungal communities, by providing inorganic nutrients, growth supplements, and trace minerals, as well as other environmental factors like pH, temperature, and oxygen. Small levels of contaminants can also act as a stimulant by activating the operons for bioremediation in the enzyme. This sort of bioremediation requires nitrogen, phosphorus, and carbon, but it is not currently employed or thought to be used industrially, as far as we know.

2.1.2. Bioaugmentation

A specific bacterium is added to the wastewater as part of the bioaugmentation technique to wastewater bioremediation, and the resulting microbial growth is thought to be able to speed up pollutant breakdown. A key component of the bioaugmentation idea is the use of microorganisms that preferentially feed on contaminated locations to improve natural microbial breakdown and accelerate population expansion. It can be used to change and get rid of non-toxic microorganisms like ethylene and chloride (Cavinato *et al.*, 2017).

Most of the time, it is not possible for native or natural microbial species to quickly degrade dangerous contaminants. In order to encourage more effective and reliable breakdown of contaminants, microorganisms have been genetically modified or altered by DNA manipulation. However, despite this objective having been anticipated for decades, there are still no full-scale implementations (Janssen and Stucki, 2020), most likely as a result of challenges with the release of modified microbes on a technological, financial, and ethical level (Deeba *et al.*, 2018).

2.1.3. Biological reactors for bioremediation

The goal of using bioreactors to remediate contaminated soil and water is to convert the contaminated medium (such as wastewater that is contaminated) into less hazardous substances by fostering a series of biological reactions (Tekere, 2019). As a result of the reactors' ability to assess temperature, pH, nutrient levels, and agitation, microbial activity and hence pollutant degradation can be increased (Jesitha and Harikumar, 2018). In numerous lab and pilot bioremediation investigations for various pollutants, microbial bioreactors have been used (Pino-Herrera *et al.*, 2017). Bioreactors are the best choice for bioremediation because of their design flexibility for a variety of processes and remediation applications. The design ought to take system waste disposal, nutrition delivery, and high cell biomass growth into account. At a few underground leaky storage tanks at industrial locations, the bioreactor approach has been tested for efficiency in the cleanup of organic pollutants (Iorhemen *et al.*, 2016).

2.1.4. Phytoremediation

It is practicable, "clean and green," environmentally friendly, economical, and environmentally beneficial to utilize plants to reduce or remove inorganic and organic toxins from the environment. A bioremediation technique called phytoremediation uses specific plants (and perhaps bacteria as well) to transfer, stabilize, and/or remove pollutants from soil and groundwater. Rhizofiltration, phytodegradation, phytostabilization, phytoaccumulation, and other mechanisms are frequently included in the phytoremediation mechanism.

Typically, phytoremediation involves the removal of contaminants or their transformations into a less harmful or basic form (Saxena *et al.*, 2019). Several plants have the ability to concentrate and extract specific hazardous components from the environment, offering a long-lasting repair strategy. It is well acknowledged that phytoremediation is an economical approach for restoring the environment. There are various phytoremediation technologies available to perform bioremediation depending on the environment and types of toxins (Malla *et al.*, 2018). It has been observed that volatile organic chemicals are taken up by plants and removed from the environment. A developing form of bioremediation involving plants is phytoremediation for wastewater.

2.2 Techniques for bioremediation

Environmental toxins can be detoxified using a technique called bioremediation, which mostly uses microbial or plant enzymes. Biodegradation, which is the biotransformation or microbiological or phytodetoxification of contaminants, is a part of the notion. A

group of bacteria use the process of mineralization to change an organic contaminant into its inorganic equivalent. Co-metabolism is a method that enables microbial growth without the exogenous supply of carbon or energy. By introducing an external microbial culture that accelerates the breakdown rate or by providing nutrients to the microorganisms (biostimulation, bioremediation), bioremediation speeds up the natural microbial biodegradation process (bioaugmentation).

2.2.1 Bioremediation methods involving bacteria and fungi

Studying the impact of environmental regulations on the effectiveness of remediating xenobiotic chemicals that survive in terrestrial ecosystems for very long periods of time is a burgeoning area of inquiry. Fungi produce extracellular enzymes like laccases and ligninases and use their mycelia to colonize soil environments. Less research has been done to examine the impact of variations in the relationship between water and temperature on the effectiveness of bioremediation fungi on xenobiotic substances (Gouma et al., 2014). These disciplines of study may lead to important, game-changing discoveries.

2.2.2. Bioremediation by periphyton

Periphytons are found in surface waters and have a significant role in shaping the primary productivity of aquatic ecosystems (Zhong et al., 2020). Algae, diatoms, fungi, bacteria, protozoa, small multicellular creatures, and organic debris are all parts of the periphyton. These creatures are crucial aquatic dwellers because they control the pace of nutrient cycling and transfer between various trophic levels of food chains or foodwebs. Periphyton communities are a crucial bio-agent for restoring aquatic ecosystems because of all these reasons. Periphyton filtration is a well-known remediation technique for purifying contaminated water.

2.3 Mechanism of Microbial Bioremediation

Most bacteria use the contaminants as a source of energy or nutrients when it comes to the method of microbial breakdown of pollutants like pesticides. They break down some contaminants to obtain nutrients or energy produced when chemical bonds are broken. These initially dangerous compounds are transformed into less or harmless metabolites as a result of this microbial breakdown. Essentially, this is how microbial bioremediation works.

Enzymatic degradation has been noted to be one of the primary mechanisms used by microorganisms in the bioremediation process in the majority of reported cases (Uqab et al., 2016). Enzymes that can break down the pesticides' active components can be produced by some bacteria. The bioremediation of pesticide pollutants may be aided by these enzymes. However, a variety of enzyme groups may be necessary for the bioremediation of pesticides due to the enormous diversity of the pesticide chemistry.

Another method of bioremediation is the demobilization of pollutants. Microorganisms are able to demobilize contaminants in a variety of ways, including sorption, which is the accumulation of organic pollutants by microbial biomass, precipitation, which is the production of reduced or oxidized forms of toxic elements, and polymerization, which is the joining of organic pollutants with each other or with naturally occurring compounds in the environment. The bioremediation process may benefit from the byproducts of microbial activity. In this case, environmental changes including pH, redox conditions, and reactive chemicals produced as a result of microbial activities in the polluted environment cause harmful compounds to transition into less dangerous or harmless molecules (Bollag and Liu, 1990).

Some genes implicated in the bioremediation of pesticides have been found in the bacterial genomes, according to scientists. It has been determined that the plasmids of bacteria contain several genes important for the synthesis of catabolic enzymes linked to the microbial breakdown of contaminants. These plasmid genes significantly aid bacterial evolution in order to acquire new derivative capacities and environmental adaptations to xenobiotic substances (Gupta and Singh, 2017). Scientists are striving to create microbial strains with novel catabolic properties that can be used in bioremediation through genetic engineering techniques as a result of technical improvements (Kumar et al., 2018).

3.0. ENZYMES FROM MICROORGANISMS FOR BIOREMEDIATION

3.1 Microbial Oxidoreductases

Various bacteria and higher plants use oxidoreductases to detoxify and break down dangerous natural combinations through the oxidative-reductive coupling. Microorganisms rob hazardous substances of their life energy by degrading them through metabolic activities. These biological enzymes are responsible for transferring electrons from a natural substrate (donor) that has become depleted to another synthetic material (acceptor). Thanks to such powerful reactions as oxidation-lessening responses, the contaminants are oxidized to harmless mixtures in this manner (Khatoun et al., 2017).

The oxidoreductases detoxify a variety of synthetic organics, including phenolic, azo rings, and aniline compounds, which are present in xenobiotics or the soil environment. Fungi like *Pleurotus ostreatus* (oyster mushroom), *Trametes versicolor*, *P. chrysosporium*, and Basidiomycetes are more effective at preventing bacteria from growing in the soil. The pulp bleaching process causes lignin to partially breakdown, which results in the production of chlorinated phenolic chemicals. Therefore, bioremediation is essential for the paper and pulp sectors.

3.1.1. Oxygenases by Microbes

Oxidoreductases are responsible for adding oxygen from sub-atomic oxygen (O₂) to the reduced substrates while utilising FAD/NADH/NADPH as a co-substrate. Depending on how many oxygens were utilized to oxygenate the reactant, oxygenases are split into two groups: monooxygenases and dioxygenases. Halogenated organic compounds, such as pesticides, are a common source of substrates for the bacteria that make up the oxygenase group. They also contribute significantly to the digestion of natural mixes by enhancing their reactivity, polarity, or achieving the cleavage of the aromatic ring.

3.2 Laccases from Microbes

The main structure of laccases includes a number of multi-copper oxidases (p-diphenol: dioxygen oxidoreductase). They are made by particular types of plants, animals, and microbial organisms and they are catalyzed by the oxidation of many reduced phenolic and aromatic substrates with the sequential reduction of subatomic oxygen to water. Isoenzymes are produced as a result of different genes encoding for different laccase structural variants (Chauhan *et al.*, 2017).

Among the compounds that internal and external laccases help oxidize are ortho-diphenols, para-diphenols, aminophenols, polyphenols, polyamines, lignins, aryl diamines, and a few inorganic ions. The oxidation, decarboxylation, demethylation, and polymerization of lignin into phenols, which are ultimately used to produce humic chemicals, are all processes that include laccases. They hold great promise for biotechnological and bioremediation applications. With the exception of iodide, halides, azide, cyanide, and hydroxide impede activity. It reacts when there is nitrogen present (Arregui *et al.*, 2019).

3.3 Peroxidases from microbes

Strong oxidizing enzymes are known as peroxidases (contributor: hydrogen peroxide oxidoreductases). They also catalyze the reduction of coal to low molecular mass fractions and the oxidation of lignin, lignocellulosic materials, and other phenolic mixes to compounds devoid of hydrogen. These peroxidases can be hem or non-hem proteins that are connected to simple organic reactions in a variety of hosts. Free radicals produced by peroxide that are metabolically active and non-specific are produced because these substances (enzymes) break down polymeric materials into short-chain water-soluble particles, which in turn encourages their transportation through microbial layers for intracellular degradation. These are related to auxin degradation, plant lignin and suberin organization, cross-linking of cell divider segments, pathogen defense, or cell growth. Microbes also carry out bio-pulping, bio-bleaching, and polymerization. The diverse fungus species have numerous isoenzymes of lignin and manganese peroxidase (Bansal and Kanwar, 2013; Cocco *et al.*, 2017).

3.3.1. Microbial lignin Peroxidases

In terrestrial ecosystems, the breakdown of lignocellulose is a critical step in the carbon recycling process. During the delignification process, basidiomycetes are surprisingly stable. The white-rot and brown-rot basidiomycetes mainly release heme proteins known as lignin peroxidases through the optional digestion of lignocellulose as a component of their metabolic activities. They are very successful in destroying the woodlands (Kumar and Chandra, 2020). In comparison, the brown rot fungus is pickier. In relation to the degradation of lignin and other phenolic compounds occurs in the presence of co-substrate H₂O₂ and veratrole liquid LiP. In this process, LiP is oxidized, allowing H₂O₂ to be reduced to water and nascent oxygen by giving it an electron. LiP receives the electron it requires from veratrole liquor to return to its starting state. The result is the formation of veratraldehyde and the production of veratrole liquor.

3.3.2. Manganese Peroxidases through Microbes

The basidiomycetes organism, which breaks down lignin, contains the extracellular heme substance MnP. (Manganese peroxidase). The transformation of manganate into manganite involves numerous processes. MnP begins the reaction by employing Mn²⁺ as a substrate. Mn³⁺ is thus generated, which oxidizes various phenolic combinations. The resulting Mn³⁺ chelate oxalate rapidly diffuses into regions that the catalyst cannot reach, like lignin, or structures that are identical to them but are buried deep underground, such xenobiotic poisons, which are not accessible to proteins (Chowdhary *et al.*, 2019).

3.3.3. Microbial versatile Peroxidases

V.P. chemicals, which have an exceptionally broad substrate specificity immediately oxidize Mn²⁺, methoxybenzenes, sweet-smelling phenolic substrates like that of MnP, LiP, and HRP. V.P. is also capable of oxidizing substrates without a trace of manganese in contrast to different peroxidases. An additional feature includes the high efficiency of V.P. with both phenolic and nonphenolic lignin display dimers. Because of its high productivity, it is often desired for biotechnological industries and bioremediation.

3.4. Hydrolases

Hydrolytic enzymes breakdown chemical links between hazardous compounds to lessen toxicity. This procedure is easily capable of breaking down pesticides like carbamate, organophosphate, and oil spill. Additionally, it catalyzes alcoholysis and condensation. This enzyme's availability, non-selectivity, and high tolerance are its key benefits. There are numerous uses for extracellular hydrolytic enzymes, such as in the food business, chemical industries, biomedical sciences, and as feed additives. These include lipases, DNases, amylases, proteases, xylanases, and pullulanase. In the breakdown of biomass, the enzymes hemicellulase, cellulase, and glycosidase are particularly active (Thakur *et al.*, 2019).

3.5. Microbial Lipases

Lipase is a lipid-degrading enzyme that has been identified from microbial, animal, and plant sources. It aids in significantly reducing the amount of organic contaminants found in the polluted soil. The substantial drop in total hydrocarbon from contaminated soil was caused by lipase activity.

3.6 Microbial dioxygenases

Dioxygenases are enzymes that give their substrates a molecular oxygen boost. Because dioxygenases oxidize aromatic chemicals, they have uses in bioremediation. The enzyme dioxygenases breaks down aromatic chemicals and produces aliphatic byproducts. The enzymes that break down intradiol and extradiol use Fe (III) and Fe (II), respectively. KP7 Nocardioideis sp. strain was discovered on a seashore near Kuwait. After an oil leak event, the dioxygenase aids in the detoxification process by degrading phenanthrene

3.7 Monooxygenases

Due to their stereo selectivity on diverse substrates, monooxygenases function as biocatalysts in bioremediation. These enzymes are utilized to break down both aromatic and aliphatic molecules and utilise substrates as the reducing agent. The enzyme methane monooxygenase is primarily involved in the breakdown of hydrocarbons.

3.8 Tyrosinases

Wastewater containing phenolic compounds is discharged by industries involved in coal conversion, the production of plastics and resins, and petroleum refining (Lee *et al.*, 2013). Phenol toxicity has an impact on the biological treatment process, or activated sludge process. Since the 1980s, when scientists started using horseradish peroxidase to oxidize phenol, enzymatic-based methods have been proven to be effective⁴³. Tyrosinase from the thermophile Symbiobacterium sp. SMH-1 demonstrated the enzyme's potential for bioconverting phenol from the phenolic resin manufacturing sector into a value-added product.

4. MICROBIAL ENYMES INVOLVED ORGANIC POLLUTANT REMEDIATION

4.1 Oxygenases in Degradation of Aromatic Compounds

The monooxygenases and dioxygenases subgroups of oxygenases are the two primary subgroups. The processes of desulfurization, dehalogenation, the oxidative release of the nitro group, and nitro group release are also carried out by them, as well as the hydroxylation of various aromatic and aliphatic molecules. Alkane monooxygenases, one of the most thoroughly characterized monooxygenases, catalyze the first phase of alkane breakdown. Two common alkane monooxygenases are those related to AlkB and proteins from the cytochrome P450 family. Both of these classes of enzymes can employ short-chain alkanes to produce. In addition, it has been found that the hydroxylation of long-chain alkanes is mediated by the flavin-binding and thermophilic solubility long-chain alkane monooxygenases AlmA and LadA. It's intriguing to note the abundance of alkane monooxygenases in some bacteria. Coexistence of many alkane hydroxylases was believed to complement and increase the variety of substrates, boosting the ability of microbes to use them in some specific situations (Xu *et al.*, 2020). Numerous alkane hydroxylases coexisting was thought to complement and broaden the range of substrates, improving the capacity of microorganisms to utilize them in some particular circumstances (Xu *et al.*, 2020).

The two most important aromatic pollutants are PCBs and PAHs, and the dioxygenases that hydroxylate aromatic hydrocarbons have been extensively studied and are essential for the biodegradation of a variety of environmental toxins. The benzene ring is oxidized by dioxygenase enzymes to start the aerobic metabolism of PAHs when two hydroxyl groups are added to aromatic molecules. For instance, naphthalene can be oxidized by a variety of bacteria using naphthalene dioxygenase (NDO) enzymes, such as Pseudomonas, Rhodococcus, and Mycobacterium (Kumari *et al.*, 2021).

Biphenyl is another pervasive organic pollutant that is a member of the PAHs. Biphenyl dioxygenase (BPDO) is a catalyst that is utilized by bacteria that metabolize biphenyl and certain PCBs to produce chlorobenzoic acids. Like NDO enzymes, BPDO is a three-component enzyme. Ferredoxin reductase (BphF) and ferredoxin reductase (BphG) function as an electron transfer from NADH to BphAE, the final two components (Suenaga *et al.*, 2017; Garrido-Sanz *et al.*, 2020).

4.2. Laccases Involved in the Ring Cleavage of Aromatic Compounds

Laccases are a broad-spectrum biocatalyst that can be used to break down companies and hospitals. The hydrophobicity of PAHs is often strong, which makes it challenging for bacteria to digest them. Laccases can, however, be used to hasten the oxidation of PAHs with a few redox mediators. During the catalyzation phase, the synthesis of aryl radicals from PAHs comes after the creation of quinones.

Previous research showed that the catalytic activity of laccases can be significantly enhanced by the addition of additional copper during the PAHs oxidation process. However, Zeng *et al.* found that the Bacillus subtilis laccase CotA may oxidize PAHs in a copper-independent way with higher laccase activity, indicating that CotA may be a potential option for PAH remediation (Zeng *et al.*, 2016).

There is evidence that laccases can break down phenolic compounds. Phenoxy radicals can be produced by the rapid oxidation of phenolic substances using laccase, followed by phenolic coupling or oxidative coupling using either C-O or C-C bonds. Notably, the reaction products of laccase-catalyzed oxidative coupling are often insoluble and easily separated by sedimentation, with some of them having polymeric characteristics. Generally speaking, laccases initiate the oxidation and polymerization of phenolic compounds to produce inert, harmless polymers, which represents a promising ecologically friendly bioremediation method. Using these methods for the breakdown process, such as phenoxy-radical mediated coupling, phenolic compounds can be eliminated

4.3. Hydrolytic Lipases/Esterases Involved in Bioremediation

The hydrolysis process is a vital step in the detoxification of contaminants. Hydrolytic enzymes called esterases and lipases are able to break the ester bond of obstinate pollutants, hence lowering their toxicity. Due to this characteristic, lipases and esterases have a significant potential for the biodegradation of organophosphate, pesticides, and plastic waste. Aryloxyphenoxy propionate (AOPP) herbicides are among the most efficient herbicides used in agriculture. These include fenoxaprop-ethyl (FE), cyhalofop-butyl (CB), haloxyfop-R-methyl (HM), quizalofop-p-ethyl (QE), and clodinafop-propargyl (CP).

Fenoxaprop-ethyl is converted into fenoxaprop acid by cleaving the ester bond in the first stage of FE biodegradation, which is mediated by the FE hydrolase Feh from *Rhodococcus* (Hou *et al.*, 2011). Feh can also convert CB, HM, and QE into their respective acids. Cyhalofop-butyl (CB) is converted to cyhalofop acid (CA) by ChbH, a second esterase found in *Pseudomonas azotoformans* QDZ-1 (Nie *et al.*, 2011). The amido bond cleavage for amide herbicides such as propanil, propham, and chlorpropham is catalyzed by arylamidase AmpA, which was isolated from *Paracoccus* sp. FLN-7 (Zhang *et al.*, 2012).

Insecticides known as pyrethroids are frequently used in residential areas and agricultural settings to control insects because of their high efficacy and minimal toxicity to mammals. Several pyrethroid-degrading enzymes have so far been cloned and investigated, and they can all transform PytY, PytH, EstP, Sys410, and other pyrethroid insecticides. By random mutagenesis and secretory expression of Sys410, a mutant enzyme with improved activity and thermostability was created. This enzyme was capable of degrading a wide range of pyrethroids and exceeded a 98% hydrolysis rate. But none of the enzymes that have been previously described can consistently and effectively break down pyrethroids.

Pesticides with an ester bond, such as pyrethroids, are related to organophosphate pesticides (OPs), which make up the majority of pesticides and more than 30% of the worldwide pesticide market. During the breakdown of OP, the phosphorus-ester (P-S) bond is predominantly hydrolyzed. The most well-known bacterial enzymes for OPs metabolism are the Opd and its homologs, which are typically grouped as phosphotriesterases (PTEs) and belong to the amidohydrolase superfamily. Numerous other types of Oph have been described in the literature thus far, including opd, opdA, opdB, ophc2, hocA, adpB, and others.

4.4. Heavy Metal Transforming Enzymes

Heavy metals are defined as substances with densities more than 5 g/cm³ that either originate naturally or are the result of human action. The heavy metal mercury (Hg) is one of many that are extremely dangerous (Verma and Kuila, 2019). In order to adapt to environments with high mercury concentrations, microbes have developed incredibly complex specialized resistance systems. One of the well-known bacterial systems that resist mercury is the mer operon, which changes ionic mercury (Hg²⁺) into the volatile elemental form (Hg⁰).

The mer operon is a gene cluster that transports and transforms both inorganic and organic mercury. It is made up of several interconnected genes. A typical mer operon's organomercurial lyase (MerB) carries out the demethylation process by cleaving the methyl group into methane (CH₄) and Hg(II), which is then converted to the volatile form by another mercuric reductase (MerA). The inner membrane-spanning proteins MerT/C/E/F/G carried Hg²⁺ to the cytoplasm, where MerA further decreased it. This process only occurs in the aerobic prokaryotes *Geobacter*, *Staphylococcus*, *Pseudomonas*, etc.

Additionally, the commencement of the mer pathway demands extremely high Hg concentrations (frequently micromolar), which are irrelevant in the bulk of naturally occurring Hg-contaminated situations since Hg or CH₃Hg⁺ concentrations normally range from picomolar to nanomolar (Lu *et al.*, 2016). The model methanotroph *Methylosinus trichosporium* OB3b is used by Lu *et al.* (2017) to describe a novel CH₃Hg⁺ demethylation process by methanotrophs, which may destroy Hg at low doses. The traditional mer pathway, in which CH₃Hg⁺ was first bound to methanobactin before the C-Hg was split by methanol dehydrogenase, differs dramatically from methanotrophic-mediated CH₃Hg⁺ breakdown.

Lead (Pb) pollution is regarded as one of the most serious environmental toxins, along with Hg pollution. This has motivated researchers to look at the many techniques microorganisms utilize to maintain their lead resistance. We pay special attention to the Pbr system, which combines efflux and precipitation in a novel way. The pbrUTR and pbrABCD transcription units are typical divisions of the pbr operons. This Pb resistance is produced in conjunction with the pyrophosphate phosphatase encoded by PbrB, which particularly promotes Pb resistance, and the P-type ATPase PbrA, which non-specifically exports Zn²⁺, Cd²⁺, and Pb²⁺. With the aid of the inorganic phosphate produced by PbrB, PbrA is able to extract Pb²⁺ from the cytoplasm and sequester it in the periplasm as a phosphatase salt (Sharma *et al.*, 2017)

Increased significantly, the hazard of arsenic (As) pollution causing cancer has garnered more attention (Deng *et al.*, 2020). Arsenate (AsV) and arsenite (AsIII), the two primary inorganic species, are more toxic and mobile than AsV. The As metabolic pathways that have been most completely studied are the ars, aio, and arr operons. Microbes have developed a number of defense mechanisms

against As toxicity (Andres and Bertin, 2016). To give a brief overview of the system, the *asrC* gene codes an arsenate reductase for the conversion of As(V) to As(III), and the *asrB* functions as an As(III) expulsion pump, allowing its cellular extrusion (Han *et al.*, 2019). The *aiO* and *arr* systems have been shown to use arsenic during metabolic activities.

4.5 Advantages of enzyme-based technology in bioremediation

The enzymes made by extremophilic bacteria have a wide range of applications because of their stability and biodegradation potential. One of the most important biotechnological applications is the bioremediation of various pollutants and dangerous compounds from water and sediments. Halophilic bacteria's extremozymes have been used in this process (Dumorne *et al.*, 2017). These microorganisms are helpful for eliminating pollutants from poor environmental conditions. Extremophilic bacteria are capable of metal biosorption under extreme environmental stress due to the structure and functional characteristics of their cell wall, capsule, Slayer proteins, extracellular polymer substances (EPS), and siderophores.

The main advantages of enzymes include their high degree of specificity, great stability at high temperatures, pH, salt, and metal concentrations, etc., as well as their quick reaction speed that reduces processing costs. The enzymes have catalytic activity, can access substrates through pores of different sizes, and can work under a variety of environmental conditions (Quiquampoix *et al.*, 2002). Enzymes speed up chemical reactions by lowering their activation energy. Extremophilic enzymes are advantageous because they can perform reactions at high temperatures, are resistant to a variety of organic solvents, and can be overexpressed in the appropriate host-vector.

Due to their selectivity and versatility, extremozymes can be used in a variety of ways during the bioremediation process to remediate wastewater. The proteases, cellulases, pectinases, keratinases, lipases, esterases, catalases, peroxidases, and phytases are among the enzymes that make up extremozymes. Amylases, pullulanases, xylanases, and phytases are also included (Schiraldi and De Rosa, 2016). Extremozymes and their application in bioremediation have received more attention in recent years. Extremozymes are more effective when subjected to genetic and chemical manipulation, as well as immobilization methods that boost their activity and stability for use in a variety of fields.

5. CONCLUSION

The dispersion of resistant residual pollutants in the environment is influenced by a variety of biological processes, including phytoremediation, biosorption, and microbial biodegradation. As concern over the harmful effects of environmental pollutants has grown in recent years, research into various techniques that can be utilized to clean up the contaminated environment has considerably increased. Only recently have the natural resources available for bioremediation been fully comprehended and utilized. We can now describe new bacterial strains, enzymes, and metabolic pathways involved in the degradation of pollutants by bacteria. Many of the enzymes that accomplish this naturally have the capacity to destroy pollutants, but they also possess special qualities and have bright prospects as biocatalysts. As a result, a wide range of disciplines, including the production of industrial biosensors, pharmaceutical development intermediates, medical bioremediation, etc., have successfully adapted a number of microorganisms and the related degrading enzymes. These programs not only promote wise use of biological resources, but they also have favorable social and economic effects that are noteworthy. Only a small part of these bacteria and the enzymes they produce to break down pollutants are understood and utilized, though.

The microbial bioremediation procedure has recently been found to be the most effective way to remove and detoxify pollutants from the environment. Future uses of bioremediation technology have a great deal of potential because they can be combined with many conventional and traditional treatment methods to entirely eliminate a range of environmental problems. It is sustainable to manage, control, and lessen environmental pollution in this way. Much study and research must be done to establish the perfect circumstances that permit the best interaction between bacteria and the contaminants in order to fully detoxify the harmful and deadly pollutants and restore the desired environmental quality. Additionally, in order for this method to be successful and long-lasting, appropriate tools for monitoring the performance of the microbes must be developed.

Therefore, bioremediation is the environmentally safe elimination of all hazardous substances from the environment using only natural, sustainable techniques. This cycle's objective is to support nature's rejuvenation through the use of existing resources and any necessary modifications. Using enzymes present in bacteria, chemicals can be eliminated. Utilizing biological systems has an advantage over utilizing non-biological ones since it prevents the production of hazardous byproducts, which is prevalent when using non-biological systems. After treatment, the proteins are processed by the local microbes in the environment. It is feasible to make catalysts on a larger scale, with better security and action, and at a lower cost with the aid of recombinant-DNA innovation, which does not require the full culture. Thus, customization is aided.

The catalyst execution for in situ bioremediation of contaminated soils and groundwater, however, may be impacted by a number of issues. Solvency, transport, adsorption, scattering, unpredictability of toxin mixtures, discovery, assurance, and verification of toxins, science, material science, and microbiology of groundwater and soil, hydrogeology, and hydrology of the degraded site, confinements of ecological gauges for water and soil, ecological conditions, supplemental sources, and proximity of electron acceptors, and primarily the biodegradability of contaminants are among them.

Rapid advancements in a number of pathways, agents used by microorganisms to deteriorate contaminations, have increased our understanding of their tools. Pathways have also been described to construct maintainable bioremediation systems for contaminating mixtures. However, it is still essential to have a full understanding of managerial viewpoints, subatomic research, and protein structure.

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