THE USE OF LACCASES IN THE BIOREMEDIATION OF AGRICULTURAL SOILS: A SUSTAINABLE APPROACH FOR THE FUTURE
/
UTILIZAREA LACAZELOR ÎN BIOREMEDIEREA SOLURILOR AGRICOLE: O ABORDARE DURABILĂ DE VIITOR

Mariana IONESCU[1)], Mariana – Gabriela MUNTEANU*[1)], Bianca – Ștefania ZĂBAVĂ[1)], Georgiana MOICEANU[1)], Mirela - Nicoleta DINCĂ[1)], Elena - Valeria VLĂDUȚ[1)], Gigel PARASCHIV[1)], Mariana FERDEȘ[1)]
[1)] National University of Science and Technology POLITEHNICA Bucharest / Romania;
Tel: +40767236522; E-mail: mariana.munteanu@upb.ro

DOI: https://doi.org/10.35633/inmateh-77-87

Keywords: *laccases, soil pollution, bioremediation, agricultural soil, sustainable remediation*

ABSTRACT
Agricultural soil contamination is one of the major global environmental problems, with direct effects on ecosystem quality, agricultural productivity, and human health. Among the methods for remediating contaminated soils, bioremediation is one of the most economical and environmentally favourable innovation. Laccases, enzymes produced by fungi, bacteria, and certain plants, are especially valuable because they can degrade a wide range of hazardous organic pollutants by simple oxidation reactions. This review summarizes the bioremediation concept, the most recent developments in soil bioremediation, and the environmental importance of this concept. Also, the article presents the action mechanisms of laccases, the life cycle analysis for bioremediation systems with laccases, providing a comprehensive overview for future developments.

REZUMAT
Contaminarea solului agricol este una dintre principalele probleme de mediu la nivel mondial, cu efecte directe asupra calității ecosistemului, productivității agricole și sănătății umane. Dintre metodele de remediere a solurilor contaminate, bioremedierea este una dintre inovațiile cele mai economice și mai prietenoase cu mediul înconjurător. Lacazele, enzime produse de ciuperci, bacterii și anumite plante, sunt deosebit de valoroase deoarece pot degrada o gamă largă de poluanți organici periculoși prin simple reacții de oxidare. Această lucrare rezumă conceptul de bioremediere, cele mai recente evoluții în domeniul bioremedierii solului și importanța ecologică a acestui concept. De asemenea, articolul prezintă mecanismele de acțiune ale lacazelor, analiza ciclului de viață pentru sistemele de bioremediere cu lacaze, oferind o imagine de ansamblu cuprinzătoare pentru cercetările viitoare.

INTRODUCTION
Lately, population growth, technological progress, intensive industrial activities, and the continuous development of synthetic compounds have led to an increase in the levels of hazardous contaminants that are affecting the environment, accumulating in the soil, water, and air *(Romero-Martínez et al., 2024; Bonnet et al., 2025*). Moreover, industrialization has led to an increase in the quantity and diversity of chemical substances in the environment, many of which are absorbed and retained by the soil for long periods of time until they are treated *(Sridharan and Krishnaswamy, 2021)*. Soil contamination with chemical substances represents a serious threat, and the detection and monitoring of new and emerging pollutants is becoming a priority, given their impact on the environment and human health. Emerging contaminants include a wide range of chemicals resulting from human activities, such as domestic, agricultural, industrial, and medical activities *(Lee et al., 2024; Oprea et al., 2009).* Contamination changes the structure of the soil, which can become unsuitable or even abandoned when pollutant levels exceed the permissible limit. Depending on their nature, pollutants are biodegradable or non-biodegradable *(Sridharan and Krishnaswamy, 2021)*.

According to The Food and Agriculture Organization (FAO), agricultural soils can be contaminated with various compounds originating from both direct sources (such as the application of pesticides and fertilizers) and indirect sources (such as flooding or atmospheric deposition) *(FAO, Sources of soil pollution).* Figure 1 shows the main sources of agricultural soil pollution.

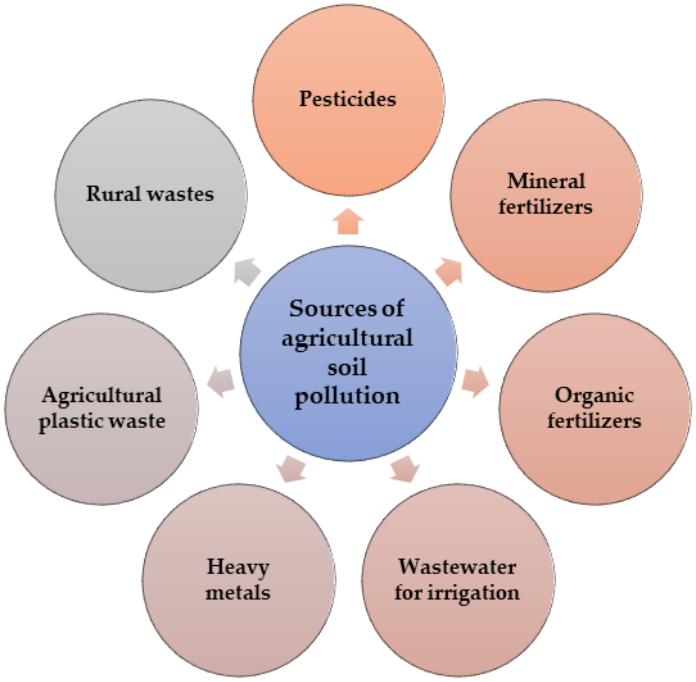

Fig. 1 - The main sources of agricultural soil pollution
(FAO, Sources of soil pollution)

For example, between 1990 and 2022, the global use of pesticides in agriculture steadily increased, reaching 3.69 million metric tons in 2022. The increase in global pesticide use raises concerns about their impact on the environment. These substances can have harmful effects on both human health and animal species, while also contaminating soil, water, and vegetation *(Dokua D., 2025).*

Thus, the excessive use of pesticides and fertilizers in agriculture has led to soil pollution with heavy metals, which has resulted in lower crop yields and reduced food quality *(Su et al., 2014; Pereira-Silva et al., 2025)*. Moreover, agricultural soil pollution also hinders the achievement of the Sustainable Development Goals (SDGs), such as goal 2, *Zero hunger* and goal 12, *Responsible consumption and production (https://sdgs.un.org/goals).* Identifying and managing sources of pollution, as well as implementing the necessary preventive measures to protect people and the environment, are essential issues addressed at a global level *(Nuruzzaman et al., 2025)*. Therefore, modern and sustainable methods of remediating contaminated agricultural soils must be developed. The bioremediation strategy uses microorganisms to break down or remove dangerous pollutants from the environment, turning them into less harmful or non-toxic compounds *(Fouad et al., 2022)*.

Conventional physical and chemical remediation methods often generate significant amounts of chemical waste and involve high costs. In comparison, biological methods represent economical and environmentally friendly alternatives to traditional methods and are therefore essential for protecting ecosystems and public health *(Abu -Tahon et al., 2025)*. Unlike conventional physico-chemical methods, bioremediation strategies are considered a more attractive alternative due to their potential to remove emerging pollutants from waste streams in an economical and sustainable manner (*Bonnet et al., 2025).*

In recent years, enzymes have gained significant importance in industry, and laccases are among them, being widespread in nature. They represent one of the oldest and best-studied enzyme systems *(Shraddha et al., 2011).* Laccases, which belong to the multicopper oxidase family, are versatile enzymes that can degrade a range of organic pollutants, including pesticides, polycyclic aromatic hydrocarbons, and pharmaceuticals products. Laccases (EC 1.10.3.2) are found in bacteria, fungi, and plants (*Bonnet et al., 2025; Bhatt et al., 2023*). Laccases have numerous applications in various industrial and technological fields, the most significant being *(Mate and Alcalde, 2015)*: soil bioremediation, biofuels, food industry, paint industry, textile industry, paper industry, biomedical industry, cosmetics industry and organic chemistry.

This review summarizes the bioremediation concept, the most recent developments in soil bioremediation, and the environmental importance of this concept. Also, the article presents the action mechanisms of laccases, the life cycle analysis for bioremediation systems with laccases, providing a comprehensive overview for future developments.

PRINCIPLE OF BIOREMEDIATION

Introduction to bioremediation

Soil is the fundamental environmental component that forms the ecosystem and serves as the material foundation for human society's industrial and agricultural output, as well as daily life *(Anae et al., 2020)*. However, soil is also a natural resource that is hard to replenish. Additionally, soil is crucial for preserving natural balance and safeguarding human health and food safety *(Lv et al., 2022).* The ecosystem, especially the soil, has become contaminated as a result of increased global industrialization and intensive farming caused by the growing human population *(Alori and Fawole, 2017).* Soil contamination occurs when contaminants generated by human activity enter the soil ecosystem through a variety of channels and their concentration overcomes the soil's ability to accommodate and assimilate them *(Li et al., 2018).* Changes in the fundamental physical and chemical characteristics of soil can result from this phenomena. Additionally, these modifications could cause the soil's natural environment function to become unbalanced and deteriorate *(Wu et al., 2018; Papageorgiou et al., 2021).*

Remediation is the process of eliminating harmful compounds from the environment or substituting them with less dangerous ones *(Uloaku et al., 2022).* Currently, various methods for soil remediation have been developed and applied. Among the factors that affect the applicability of remediation method are the remediation efficiency, contamination type, and cost-effectiveness. Previously, excavation, landfill disposal, and waste treatment techniques (like incineration and inertisation) were used for remediation of contaminated soils. In recent decades, the understanding of remediation methods has developed rapidly. Risk assessment is now the foundation of soil remediation procedures, and several treatment methods seems suitable at the field scale *(Santos et al., 2025).*

Remediation technologies can be classified into two major categories, *in situ* and *ex situ*. The *ex situ* category includes the remediation methods which involve the excavation and transfer of contaminated soils for treatment. Ex situ technologies include the added cost of excavation and transportation, but they are typically controlled better and involve less time. Ex situ treatments often cause the soil's organic matter and structure to deteriorate, making restoration more difficult, expensive, and time-consuming. By *in situ* remediation methods, the soil pollutants are removed right at the pollution site *(Romantschuk et al., 2023).* The *in situ* methods sometimes involve some soil manipulation in order to add compounds that will accelerate the remediation process. The most significant benefit of *in situ* treatment is that it makes it possible to treat soil without the need for excavation and transportation, which could result in significant cost savings and less environmental effects *(FAO and UNEP, 2021).*

Another classification of remediation technologies is made by taking into account the type of operation on which the process is based. Thus, according to the United States Environmental Protection Agency (USEPA) remediation techniques can be categorized into biological, chemical, physical, and thermal methods *(Uloaku et al., 2022)*. Figures 2 and 3 present the categories of *in situ* and ex situ technologies used for soil remediation.

Bioremediation techniques

Biological remediation has received a lot of interest lately because it is considered to be a more environmentally friendly option given the negative effects linked to other techniques *(Dincă et al., 2025).* Biological (nature-based) remediation methods involve the use of soil-dwelling organism (microorganisms such as bacteria and fungi, macroorganisms, or plants) to degrade the pollutants. A biological remediation strategy can frequently combine more than one method, to improve the contaminants biodegradation *(FAO and UNEP, 2021)*.

Bioremediation represents the process of using microorganisms (bacteria, fungi, algae, and plants) to break down, modify, immobilize, eliminate, or detoxify different physical and chemical contaminants from the environment. The enzymatic metabolic pathways of microorganisms accelerate the biochemical reactions that degrade contaminants *(Nannipieri et al., 2002; Khalid et al., 2021)*. Microorganisms must come into contact with substances that give them the energy and nutrition they require to grow and multiply in order to combat contaminants *(Garg et al., 2012)*.

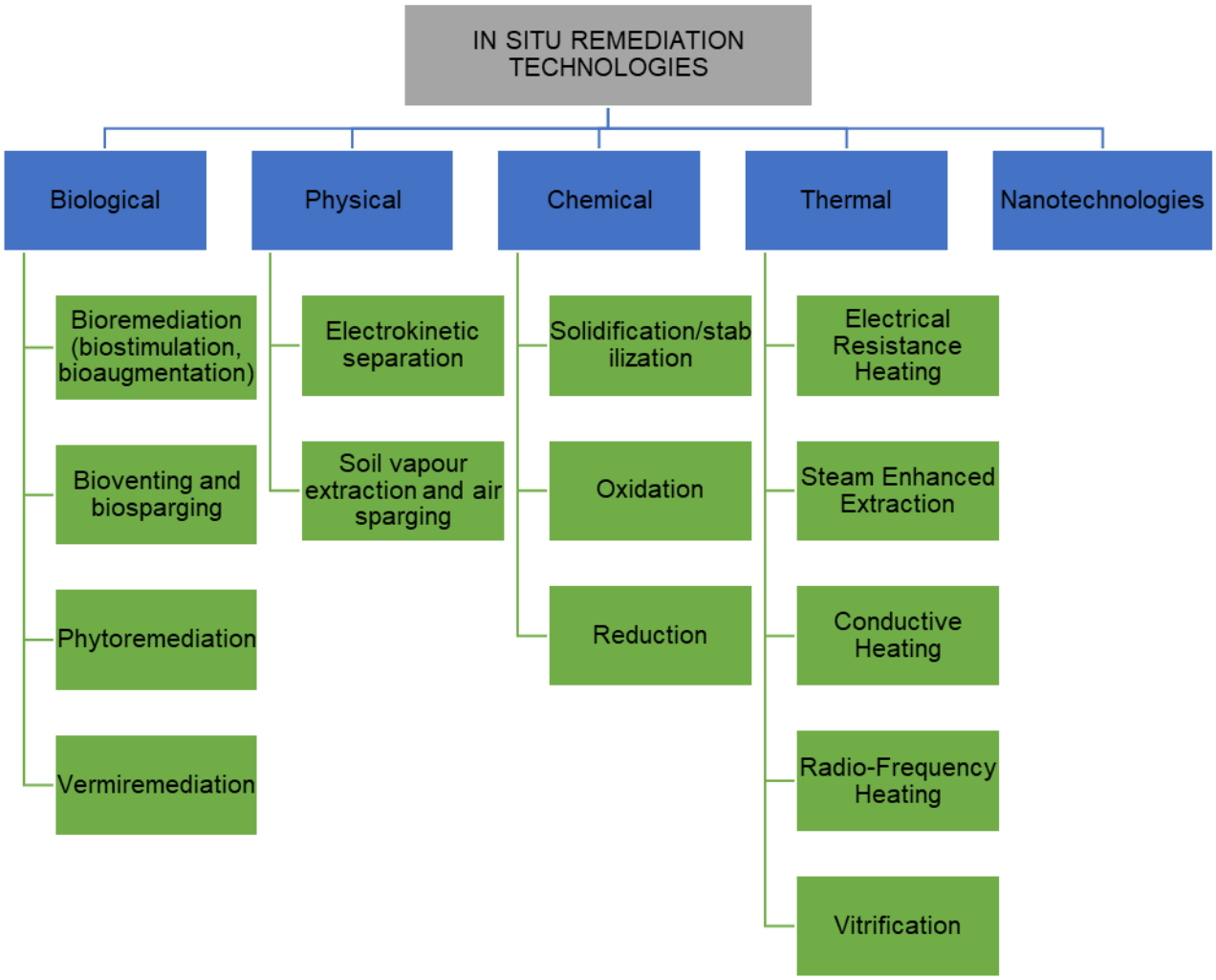

Fig. 2 - *In situ* remediation technologies of soil
(adapted after FAO and UNEP, 2021)

Bioremediation is one of the treatment techniques that has gained significant attention as it is an efficient, economical and environmentally friendly technique *(Feng et al, 2021).* Several factors must be taken into account when choosing the appropriate method of bioremediation, including: pollutant type and concentration, affected area, remediation method cost, depth of contamination, oxygen concentrations, amount of nutrients, ambient temperature, soil properties, soil moisture and pH, environmental policies (*Koshlaf and Ball, 2017; Alori et al, 2022; Micle and Sur, 2021*). Figure 4 presents the most used methods of bioremediation, classified as *in situ* and *ex situ* methods.

Ex situ bioremediation

Biopiling represents a bioremediation method widely utilized for ex situ remediation of soils contaminated by a variety of pollutants. In biopiling, the contaminated soil is dug up and placed in piles, followed by homogenizing and amending the excavated soil to improve the conditions for pollutants biodegradation by microorganisms. Biopiles, named also biocells, are frequently built and handled similarly to a compost windrow. Microorganism's activity can be increased aerobically or by supplying nutrients and minerals, and modifying pH or moisture *(Germaine et al., 2015).* Extreme air temperatures can slow down bioremediation by drying out the soil and increasing the probability that it will evaporate rather than be broken down by microorganisms *(Ojha et al., 2021).*

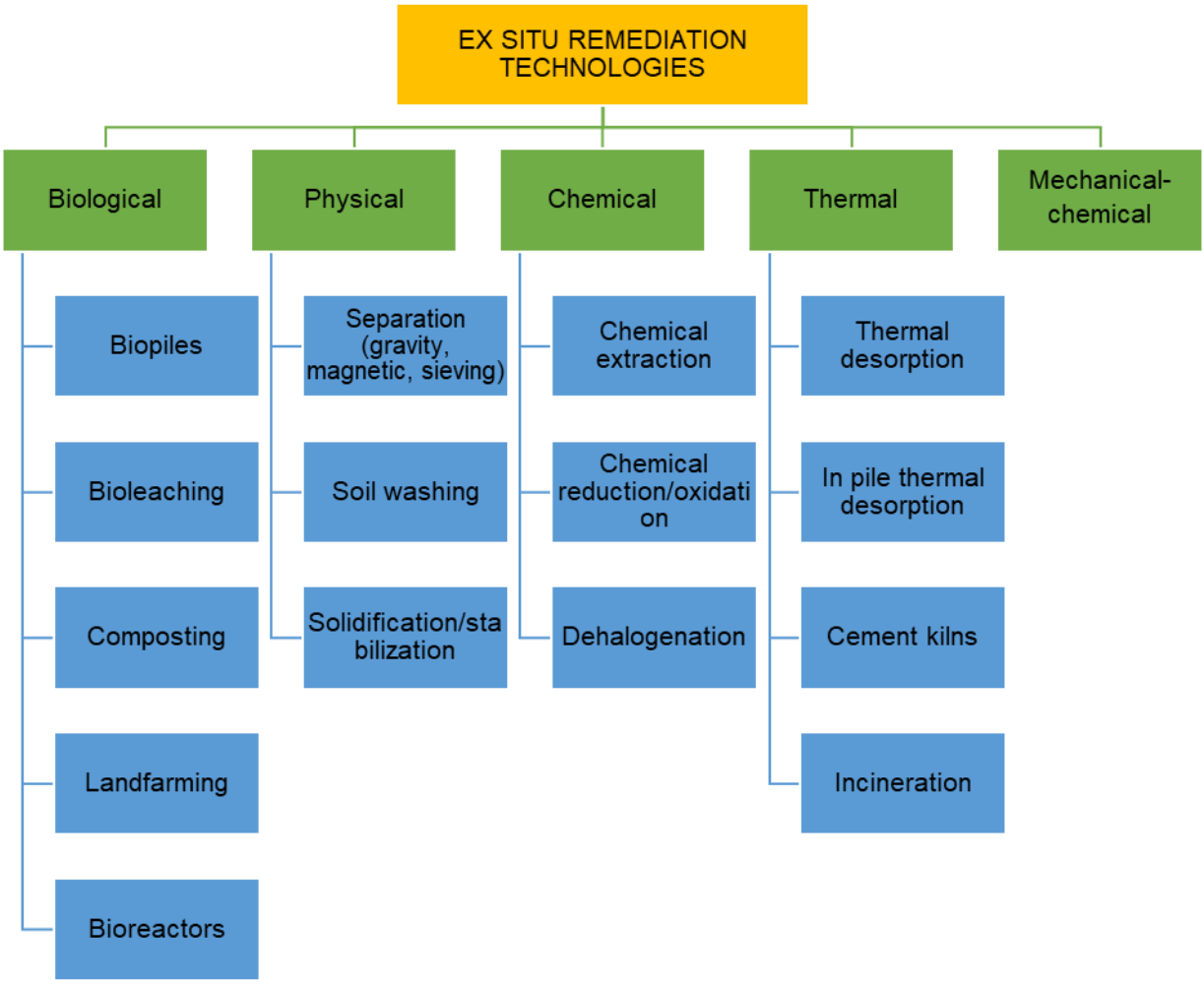

Fig. 3 - Ex situ remediation technologies of soil *(adapted after FAO and UNEP, 2021)*

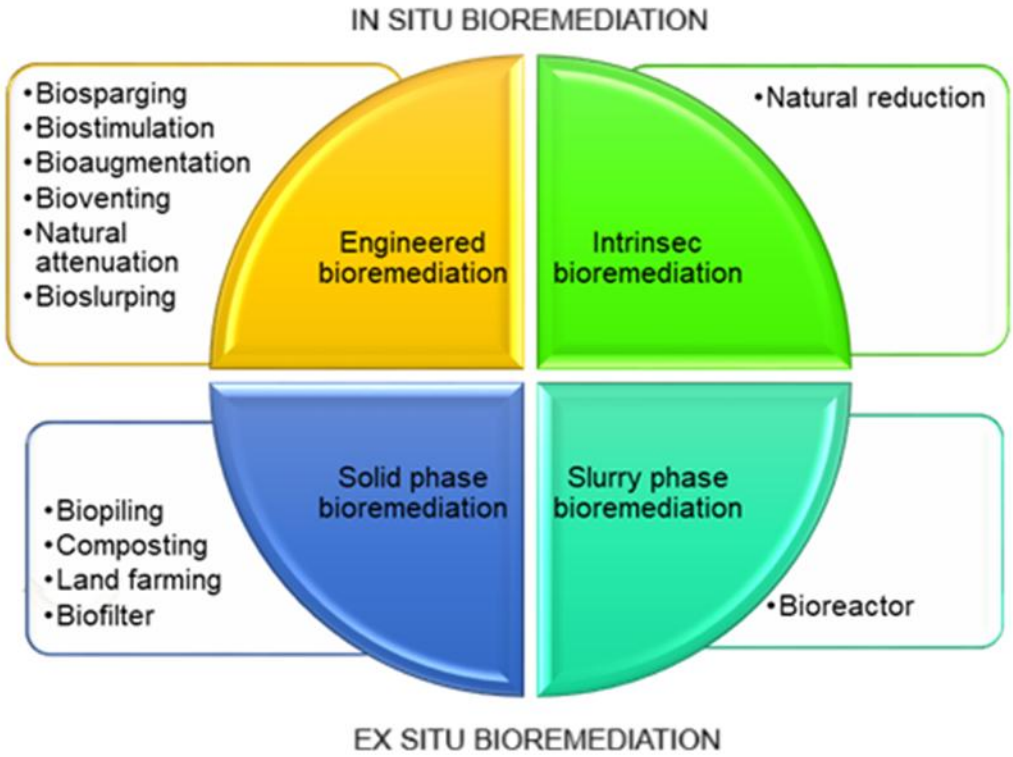

Fig. 4 – Bioremediation techniques
(adapted after Bala et al., 2022)

Similar to biopiling**, composting** is an aerobic ex situ technique that mixes the excavated contaminated soil with organic additions (such as straw or green waste) and a non-hazardous bulking agent to confer on microorganisms the ideal amounts of moisture and oxygen. The mixture typically consists of 75% contaminated soil and 25% organic fraction. However, this can be modified depending on different soil types, pollutant concentrations, and other factors. In order to improve the composting process, the pile may be mechanically stirred to maintain aeration, homogenize the mixture, and provide uniform biological activity across the pile *(Fitri et al., 2017).*

Land farming represents a bioremediation method that is widely spread due to its low operating costs, process simplicity, absence of special equipment, and small ecological footprint *(Janssen and Stucki, 2020; Wang et al., 2021*). This method assumes that excavation of polluted soil, followed by the transportation to the land farming site and the soil spreading in a thin layer on an impermeable surface or a biologically active land. Sometimes organic amendments (such as manure or sewage sludge) are added to the contaminated soil before it is ploughed into the soil's surface. This approach promotes biodegradation by the natural microorganisms, aerobically stimulated by repeated ploughing, and by supplying nutrients to support microorganism growth *(Patel et al., 2022).*

Bioreactors are equipment where the polluted soil is mixed with nutrients to stimulate the microorganism's activity, resulting in the degradation of pollutants. Bioreactors can be open or closed and can operate aerobically or anaerobically, accordance with the contaminants and microorganisms involved in the degradation process. Among the advantages of bioreactors are shorter remediation times and high process controllability *(Davoodi et al., 2020).*

In situ bioremediation

Natural reduction is also known as *in situ* bioremediation. Intrinsic bioremediation involves the use of contaminated sites without requiring human intervention. This process aims to stimulate a microbial population that already exists in the respective site. The biodegradation process of contaminants, as well as those that are resistant, is based on aerobic and anaerobic reactions in microorganisms. Because this approach requires minimal external input, natural attenuation is considered a low-cost remediation method *(Gurkok, 2021; Sharma, 2019).*

Bioventing represents a method that uses regulated airflow to stimulate the activity of native microbes by supplying oxygen to the unsaturated zone. The addition of moisture and nutrients during the bioventing procedure facilitates the bioremediation process. As a result, contaminants will be converted by microbes into harmless compounds. Bioventing is a technique that stimulates native microflora by supplying sufficient aeration, thereby enhancing microbial biodegradation and promoting the removal of heavy metal pollutants through precipitation *(Anekwe and Isa, 2021).*

Biosparging represents a bioremediation method (mostly used to remove petroleum), similar to bioventing, which involves injecting air into subsurface soil in order to facilitate the degradation of pollutants. But when biosparging is used, air is added to the saturated zone, which encourages volatile organic compounds to move to the unsaturated zone, where biodegradation occurs *(Azubuike et al., 2016).*

Natural attenuation is an *in situ* treatment technique that lowers the concentration of pollutants and stops the spread of pollution from chemical spills by utilizing natural processes. Depending on whether the method eliminates the contaminant or only lowers its concentration, it can be classified as either destructive or non-destructive. Natural attenuation is a proactive method that focuses on confirming and monitoring natural remediation processes rather than only depending on manufactured procedures *(Ebuehi et al., 2005; Declercq et al., 2012)*.

Bioslurping represents a multiphase extraction technique that combines elements of bioventing, vacuum-enhanced extraction, and free-product recovery to remove light non-aqueous phase liquids (LNAPLs), such as gasoline or diesel, while stimulating biodegradation of residual hydrocarbons in the subsurface *(Anekwe and Isa, 2021)*. The bioslurping method presents the advantage to reduce the costs related to storage, processing and disposal, but the disadvantage is that microbial activity, O_2 transfer rate, and air permeability are all decreased by excessive soil moisture.

Biostimulation is a process that optimizes environmental conditions through the addition of limiting nutrients, biopolymers, or biosurfactants, to enhance microbial growth, thereby increasing metabolic activity and accelerating contaminant degradation. Biostimulation is one of the most widely used bioremediation techniques, owing to its low cost and high efficiency.

As disadvantages of biostimulation, the long period of treatment and the limited number of microorganisms that can totally mineralize the contaminants can be mentioned *(Sales da Silva et al., 2020; Lopes et al., 2022).*

The bioremediation method named **bioaugmentation** is a technique of improving microorganism populations' performance by introducing genetically modified bacteria, cultivated bacterial isolates, or consortia enriched with particular catabolic activities to accelerate the contaminants degradation. Through the bioaugmentation technique, pollutants that are resistant to the indigenous microbiota are degraded by exogenous microorganisms. Although bioaugmentation is a non-invasive and relatively rapid method, it has the disadvantages of high energy consumption and is often considered to result in incomplete remediation *(Adams et al., 2020; Myazin et al., 2021).*

LACCASES – STRUCTURE, PROPERTIES, AND MECHANISM OF ACTION

Laccases are a class of multicopper oxidoreductase enzymes that catalyse the oxidation of a wide range of aromatic and non-phenolic compounds, while simultaneously reducing molecular oxygen to water. These enzymes are found in numerous organisms, from plants and fungi to bacteria and some insect species, and play an important role in biological oxidation and detoxification processes *(Jones and Solomon, 2015).*

From a biochemical point of view, laccases show great diversity in terms of temperature and pH stability, specific activity, and range of oxidizable substrates. They can act on phenolic compounds, aromatic amines, synthetic dyes, as well as persistent organic pollutants. In addition, some enzyme forms can be stabilized and enhanced through the use of redox mediators, thereby extending their oxidation capacity *(Hilgers et al., 2018).* Therefore, studying the structure, properties, and mechanism of action of laccases provides a fundamental scientific basis for the development of sustainable technologies that harness natural oxidation processes to reduce the chemical impact on the environment *(Brugnari et al., 2021).*

Laccases produced by fungi, bacteria and plants present different molecular structure, redox potential and environmental adaptability, making them suitable for soil bioremediation *(Baldrian, 2006; Giardina et al., 2010; Janusz et al., 2020)*. In their natural environment, microorganisms can produce laccases during substrate degradation, as well as in the processes of protection, virulence and pathogenicity, sporulation, and pigment synthesis. The most important bacteria that produce laccases are those included in the Firmicutes, Cyanobacteria, Proteobacteria, Actinobacteria, Aquificae, Deinococcus-Thermus, Archaea phyla, and some human pathogens. Bacterial laccases can be synthesized extracellularly or intracellularly, during the exponential growth phase, and when a certain substrate is present. Fungal laccases are mostly synthesized by fungi from Basidiomycota, Ascomycota, Zygomycota, Chytridiomycota and Oomycota phyla. Fungal laccases usually present a higher redox potential than the bacterial laccases and are well known for their ability to breaking down lignocellulosic material. Also, fungal laccases are involved in the decomposition of different substrates, including phenolic compounds, aromatic amines, polycyclic aromatic hydrocarbons (PAHs), pharmaceuticals, synthetic dyes. Plant laccases are synthesized by various species, including pears, turnips, cabbages, apples, potatoes, asparagus, and others. Plant laccases are mainly associated with lignin metabolism but provide useful insights into enzyme behaviour in solid matrices *(Madhavi and Lele, 2009)*. In soil systems, laccase activity is dominated by solid-phase catalysis, where enzymes interact with pollutants bound to soil particles, emphasizing the role of enzyme adsorption and redox mediation *(Morozova et al., 2007; Paraschiv et al., 2022)*.

The importance of laccases for the environment

In the context of rapid urbanization, anthropogenic activities, industrial expansion, and economic growth have intensified the exploitation of natural resources, leading to significant challenges in waste management, disposal practices, and the emergence of various environmental pollutants. To ensure a sustainable environment, these contaminants must be efficiently removed or degraded. Microorganism- or enzyme-based bioremediation offers a promising approach, enabling the degradation and/or transformation of pollutants into less toxic or non-toxic compounds. Laccase, an oxidoreductase enzyme produced by diverse microorganisms, is particularly notable due to its broad substrate range and ability to catalyse single-electron oxidation reactions across numerous chemical structures. These characteristics make it highly effective for the degradation of chemical contaminants, including emerging pollutants. However, large-scale applications require enhanced reusability, thermal stability, and operational robustness. These demands have driven the development of strategies such as enzyme immobilization and protein engineering to obtain more resilient and efficient laccase variants *(Dong et al., 2023).*

The significance of fungi and their enzymatic systems in pollution control and environmental management have become increasingly well recognized. Among the enzymes with major environmental relevance are hydrolases, laccases, lyases, peroxidases, tyrosinases, and cytochrome P450 monooxygenases *(Kües, 2015; Martinkova et al., 2016)*.

Along with hydrolase enzymes, laccases have gained increasing interest due to their effectiveness in treating environmental pollutants and degrading household, industrial, and agricultural waste. The rising accumulation of potentially hazardous substances highlights the need to harness the biodegradative capacity of these enzymes *(Paraschiv et al., 2022).*

Because of their low substrate specificity, laccases can transform a wide range of compounds while producing only water as a by-product *(Zhang et al., 2020; Iark et al., 2019).* Consequently, they are increasingly applied in the treatment of degradation of antibiotics, dyes, agro-waste, in textile and pulp industries etc (Figure 5).

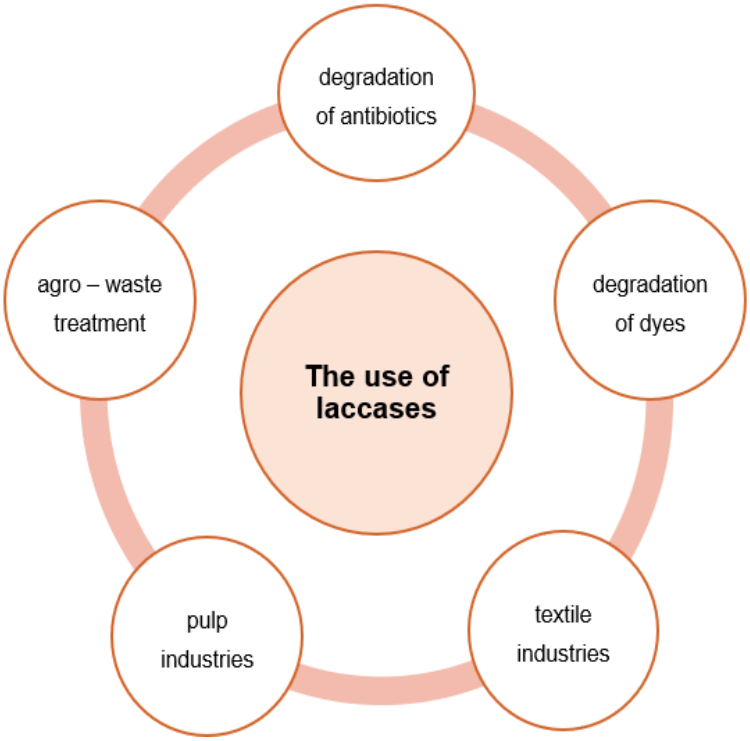

Fig. 5 - The application of laccases in the degradation of hazardous compounds
(adapted after Paraschiv et al., 2022)

Regarding antibiotic degradation, the most frequently studied classes include penicillins, tetracyclines, sulphonamides, quinolones, and trimethoprim, with particular attention given to sulfamethoxazole and tetracycline. In one study, the laccase from *T. versicolor* was used for penicillin degradation, achieving removal efficiencies ranging from 54 to 100% *(Balcazar-Lopez et al., 2016)*. The same laccase was also applied to the degradation of sulphonamides, reaching 100% efficiency after 8 h, and to tetracycline, with an efficiency of 78% after 18 h (*Rodriguez-Rodriguez et al., 2012; Llorca et al., 2015*).

The textile industry is a major contributor to global pollution, releasing approximately 280.000 tons of dyes into the environment each year *(Pierrat et al., 2023),* over 70% of which are azo dyes with carcinogenic, mutagenic, and teratogenic effects due to the stability of their azo (–N=N–) bonds, thereby intensifying the harmful impact of untreated industrial wastewater on environmental and public health *(Golka et al., 2004; Franca et al., 2020)*. Various physical and chemical methods—such as membrane filtration, adsorption, electrocatalytic degradation, and Fenton oxidation—have been applied for azo dye removal *(Hasan et al., 2020; Maiti et al., 2023*). More recently, enzymatic degradation has emerged as an eco-friendly alternative due to its low energy demand and absence of secondary pollutants. Enzymes such as azoreductase, peroxidase, and notably laccase have shown high potential for azo dye degradation *(Misal and Gawai, 2018).* However, free laccase suffers from limited stability and poor reusability, which has prompted extensive research on immobilization techniques to enhance its performance *(Kashefi et al., 2019).* Regarding the treatment of agro-waste using laccase, *Schroyen et al.* evaluated corn stover pretreatment with laccase, manganese peroxidase, and versatile peroxidase over incubation times of 0, 6, and 24 h *(Schroyen et al., 2014).* They reported that

laccase pretreatment enhanced methane production by 25%, while peroxidase pretreatment led to a 17% increase. In addition, the application of MetZyme, a novel bacterial laccase, was evaluated; when used in combination with alkali extraction, it enhanced the hydrolysis and fermentation of steam-exploded wheat straw, leading to increases of 21% and 30% in glucose and xylose concentrations, respectively *(Moreno et al., 2016).*

In the textile and pulp industries, fungal laccase is used for bio-bleaching, helping to remove synthetic dyes and inks. These compounds, which often exhibit toxicity and are difficult to break down because of their complex aromatic structures, can harm aquatic ecosystems by lowering oxygen levels and reducing microbial photosynthesis *(Cañas and Camarero, 2010).* Laccase can degrade them, but typically requires synthetic mediators such as 1-hydroxybenzotriazole (HBT), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), or violuric acid (VLA) *(Yesilada et al., 2018).*

In food processing, laccase removes phenolic compounds that cause haze and colour instability in beverages like wine, beer, and juices. It can also induce protein crosslinking through natural mediators, improving the texture, functionality, and nutritional quality of various plant- and dairy-based products *(Li et al., 2021; Manhivi et al., 2018).*

Properties of laccases

Laccases are multicopper enzymes characterised by a distinctive molecular structure. They are distinguished by the presence of three different types of copper (T1, T2, and T3) integrated into an active centre that has the ability to successfully transfer electrons. The structure of the enzyme in its active form consists of a trinuclear group composed of T2 and T3 copper, to which T1 copper is added, which is responsible for capturing electrons from the substrate. This structure allows the direct reduction of molecular oxygen to water without creating dangerous reactive intermediates such as hydrogen peroxide or free radicals. In addition, T1 copper and the transfer of charge from cysteine to the Cu (II) ion are responsible for the characteristic absorption in the 600 nm region, which gives laccase its blue colour *(Gałązka et al., 2023).*

Most fungal laccases are monomeric or oligomeric (dimer or tetramer) glycoproteins with molecular weights between 60 and 100 kDa, of which up to 50% are carbohydrates. Thermal stability, resistance to proteolytic degradation, and copper retention in the enzyme structure depend on this glycosylation. Attached monosaccharides, such as glucose, mannose, galactose, and arabinose, provide additional stability and allow for efficient secretion of enzymes into the extracellular environment. This is a characteristic of most fungal laccases *(Galhaup et al., 2002).* In terms of physicochemical properties, laccases function best at pH values of 3.5–5 for fungal forms and 6.5–7.5 for plant forms. This demonstrates the evolutionary adaptation of enzymes to their physiological environment. Intracellular laccases in plants polymerize monolignols during lignin formation, while extracellular fungal laccases, secreted in acidic environments, are mainly responsible for the decomposition of phenolic compounds in the environment *(Madhavi and Lele, 2009).*

From an evolutionary perspective, the spread of laccases across different phases of biological life indicates that this enzyme is quite old. Phylogenetic studies on multicopper oxidases show that simple structures with two cupredoxin domains, which are specific to bacterial laccases, preceded three-domain laccases, which are specific to fungi and plants. This modular derivation shows progressive functional specialization. It begins with antioxidant protection functions and engages in the remodelling of lignocellulosic materials *(Janusz et al., 2020).*

The thermal stability of laccases is influenced by a number of physicochemical factors, such as the compact organization of the protein globule, the degree of hydrophobicity, the distribution of charged residues on the protein surface, the number of intramolecular salt bridges and hydrogen bonds, as well as the presence of specific amino acid residues in higher proportions. These structural elements contribute to considerable variability in thermal stability, observed both between different species and even between distinct isolates of the same species *(Hildén et al., 2009).*

Regarding enzymatic activity, most laccases reach their optimal performance at temperatures typically ranging from 50 to 70 °C, indicating a functional adaptation to relatively warm environments. However, there are enzyme variants with different behaviour, whose maximum activity occurs at lower temperatures, below 35 °C *(Baldrian, 2006).*

Many xenobiotic chemical substances, including synthetic dyes, antibiotics, polycyclic aromatic hydrocarbons, and aromatic amines, can be oxidized by laccases. However, several substituted phenols are the usual substrates for laccases *(Giardina et al., 2010).* The quantity and type of substituents in the phenolic ring also affect the catalytic effectiveness of different substances. Compared to laccases of ascomycetes,

plants, and bacteria, laccases of basidiomycetes have a wider substrate specificity because of a larger redox potential. Additionally, mediators—low molecular weight substances that function as electron shuttles—can be used to increase the substrate specificity of laccases *(Loi et al., 2021).*

Laccases – Production microorganisms

Laccases constitute a phylogenetically diverse group whose enzymatic systems are essential for degrading natural organic matter and transforming xenobiotic contaminants. Laccase enzymes are prevalent in fungi, bacteria, and actinomycetes, underscoring their ecological significance and metabolic adaptability (*Shraddha et al., 2011; Mahuri et al., 2023)*. In recent years, researchers have focused on microbial laccases due to their strong catalytic abilities, wide range of substrates and potential for sustainable bioremediation. As agricultural soils are increasingly polluted by persistent chemicals such as pesticides, pharmaceuticals, dyes, phenolic compounds, and polycyclic aromatic hydrocarbons, studying microbes that produce laccases has become important for creating environmentally sustainable remediation methods *(Janusz et al., 2020).*

Among microorganisms, filamentous fungi, particularly white-rot Basidiomycetes, are recognized as the most abundant and efficient natural producers of extracellular laccases. Species such as *Trametes versicolor, Pleurotus ostreatus, Cerrena unicolor, Phanerochaete chrysosporium*, and *Lentinus tigrinus* are consistently identified as high-yield laccase producers because of their ligninolytic enzymatic systems, which have evolved to decompose complex aromatic polymers in wood (*Yang et al., 2017; Brijwani et al., 2010)*. These fungi secrete several types of laccases, each with different biochemical features, allowing them to oxidize a wide range of substrates. Their high redox potential, ability to secrete extracellular enzymes, and functionality in acidic environments make them highly effective for oxidative degradation processes. Such processes are crucial for soil bioremediation. The physiological functions of fungal laccases include lignin depolymerization, pigmentation, sporulation, detoxification of phenolic compounds, and protection against oxidative stress, highlighting their ecological significance (*FEMS Review, 2006; Janusz et al., 2020)*.

A notable characteristic of fungal laccases is their inducibility by environmental stress factors, including copper ions, aromatic compounds, xenobiotics, and lignocellulosic residues. Exposure to these stressors frequently upregulates laccase gene expression and enhances enzyme secretion, which can be strategically utilized in biotechnological applications *(Yang et al., 2017; Guan et al., 2025)*. In addition, solid-state fermentation with agricultural wastes such as rice straw, wheat bran, corn cobs, and sugarcane bagasse has been shown to significantly increase laccase production, providing a cost-effective and sustainable method for large-scale enzyme synthesis *(Wang et al., 2019)*. The interaction between fungal metabolism and agricultural biomass is particularly advantageous for soil remediation, as residues or composted agro-waste can serve as both a treatment medium and a substrate for laccase induction.

Although fungal laccases have been extensively studied, bacterial laccases are gaining attention for their superior physicochemical stability. Genera such as *Bacillus*, *Streptomyces*, *Pseudomonas*, *Azospirillum*, and *Ralstonia* produce laccases with unique structures that remain stable at high temperatures, alkaline pH, and in the presence of pollutants, including heavy metals and industrial dyes *(Acién et al., 2016; Chauhan et al., 2017; Elsayed et al., 2023*). The unique properties of bacterial laccases make them effective for remediation in environments where fungal enzymes may fail. For example, *Bacillus aryabhattai* TFG5 exhibits both strong laccase and tyrosinase activities, providing dual oxidative functions vital for breaking down complex pollutants *(Muniraj et al., 2022)*. In addition, *Bacillus cereus* strains from dye-contaminated effluents produce substantial extracellular laccase, which remains active against synthetic dyes and aromatic wastes, highlighting their biotechnological value in soil and water treatment *(Bhelose et al., 2024; Thangaraj et al., 2020)*.

Bacterial laccases are classified as either classical three-domain multicopper oxidases or as the more compact two-domain bacterial laccases. The two-domain bacterial laccases exhibit enhanced thermostability and resistance to denaturing agents, which makes them promising candidates for industrial-scale remediation processes *(Chauhan, 2017).* These characteristics are especially important in agricultural soils, where fluctuating conditions, such as pH changes resulting from fertilizer application or seasonal temperature variations, can hinder the effectiveness of bioremediation. Consequently, bacterial laccases provide the resilience and adaptability required to enhance bioremediation in dynamic soil environments.

Actinomycetes represent a significant microbial group capable of laccase production, yet they have received less research attention compared to fungi and common soil bacteria. Genera including *Streptomyces*, *Micromonospora*, and *Thermomonospora* display laccase activity linked to their ability to degrade complex plant materials, pigments, and xenobiotic compounds *(Goodwin et al.,2010; Fernandes et al., 2014)*.

Laccases from actinomycetes often exhibit enhanced stability under alkaline conditions, distinguishing them from fungal laccases and thereby increasing their applicability in environments with neutral or slightly alkaline pH levels. Recent investigations of actinomycetes from mangrove, marine, and terrestrial ecosystems have demonstrated their ability to degrade synthetic dyes, textile effluents, phenolic pollutants, and polyethylene-based plastics via laccase-mediated oxidative depolymerization *(Vanathi et al., 2024; Bhelose et al., 2024)*. This metabolic versatility highlights the potential for broader application of actinomycete laccases in soil remediation, particularly in sites contaminated with persistent polymers or industrial chemicals.

In addition to their natural diversity, microbial laccase producers are the focus of extensive biotechnological optimization. Advances in heterologous gene expression, protein engineering, and directed evolution have facilitated the development of recombinant laccases with enhanced catalytic efficiency or broader substrate specificity *(Guan et al., 2025*). The expression of fungal laccases in yeast, bacterial, and plant systems allows the large-scale production of enzymes with characteristics tailored for environmental applications. Similarly, engineered bacterial laccases with modified metal-binding domains or active sites can be designed to target specific agricultural pollutants, including phenoxy-acid herbicides, chlorophenols, and benzoic acid derivatives.

Microbial laccase producers also serve an ecological role. Microbial laccase producers fulfil essential ecological functions. In natural ecosystems, they contribute to carbon cycling, humification, and detoxification of phenolic allelochemicals, serving as key oxidative agents that influence soil chemistry. In agricultural soils, these activities facilitate the breakdown of persistent pollutants, including pesticide residues, aromatic hydrocarbons, antibiotics, and endocrine-disrupting compounds, which accumulate due to intensive use of agrochemicals *(Ezra et al., 2025; Yang et al., 2017)*. The enzymatic transformation of these contaminants into less toxic or more biodegradable intermediates supports the gradual restoration of soil health. Research shows that these enzymes can operate across a wide range of environmental conditions. Fungal laccases excel in acidic environments and in high-organic-matter soils; bacterial laccases are resilient to alkaline conditions, salinity, and temperature fluctuations; and actinomycete laccases tolerate a variety of pH conditions and can interact with complex xenobiotic substrates. This ecological complementarity underpins the potential for designing microbial consortia or multi-enzyme remediation systems that maximize oxidative capacity in heterogeneous soil environments.

Catalytic mechanism of laccases

Laccases operate via a specialized catalytic mechanism that sets them apart from other microbial oxidoreductases and facilitates the transformation of a wide range of xenobiotic compounds under environmentally compatible conditions. In contrast to organism specific features, the mechanism of laccase activity is determined by the enzyme's multicopper active site, which directs electron transfer, substrate oxidation and oxygen reduction. This metal-based structure enables laccases to couple the oxidation of aromatic or phenolic substrates with the four-electron reduction of molecular oxygen to water, an energetically favourable process that does not generate harmful by-products *(Solomon et al., 2014; Morozova et al., 2007*). The subsequent sections will examine the structural features of the laccase active site, the sequential electron transfer events, and the enzyme's environmental implications to clarify how the catalytic cycle provides an efficient benign oxidation pathway, thereby positioning laccases as promising agents for bioremediation applications.

The laccase catalytic mechanism is based on four copper ions organized into three spectroscopically and functionally distinct centres: type 1 (T1), type 2 (T2), and binuclear type 3 (T3). The T1 copper serves as the primary electron acceptor from reducing substrates. Its characteristic "blue copper" chromophore reflects an electronic structure that supports rapid long-range electron transfer, enabling oxidation of substrates several angstroms from the active site *(Solomon et al., 2008)*. Following electron transfer to T1, the enzyme relays the electron to the T2/T3 trinuclear cluster through a conserved pathway involving histidine and cysteine residues. At this site, molecular oxygen is completely reduced to water, a process that regenerates the oxidized copper centres and prevents the accumulation of reactive oxygen intermediates *(Baldrian et al., 2006)*.

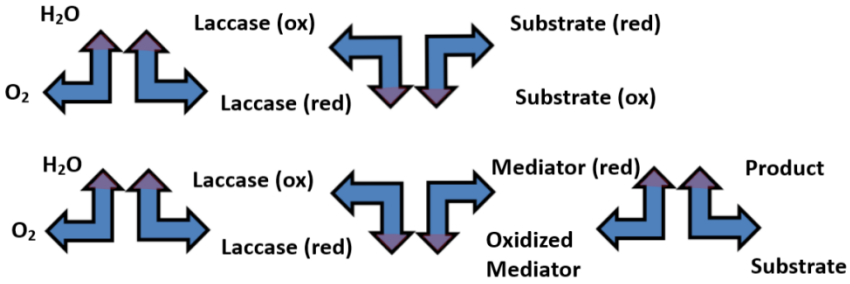

Fig. 6 - Catalytic Mechanism of Laccases
(adapted from Paraschiv et al., 2022)

Laccase activity on substrates is represented by several mechanisms. As shown in Figure 3, the simplest mechanism involves the enzyme directly catalysing the oxidation of the substrate. This direct reaction occurs when the substrate possesses and appropriate redox potential. But, if the substrate doesn't meet this requirement, oxidation can proceed via a chemical mediator (*Paraschiv et al., 2022).*

Laccase-mediated substrate oxidation typically produces reactive radical intermediates, which subsequently undergo spontaneous chemical rearrangements such as coupling, polymerization or cleavage reactions *(Giardina et al., 2010)*. The diversity of radical transformations allows laccases to target a wide spectrum of organic pollutants, including phenolic pesticides, aromatic hydrocarbons, synthetic dyes, endocrine-disrupting compounds, and pharmaceutical residues. In agricultural soils contaminated with persistent aromatic molecules, these radical-driven processes initiate detoxification by converting complex xenobiotics into more reactive or biodegradable intermediates *(Ezra et al., 2025)*. Notably, because radical formation occurs outside the enzyme's active site, laccase-mediated oxidation can impact pollutants beyond the immediate proximity of microbial cells, enabling large-scale transformation in soil environments.

While high-redox-potential fungal laccases can directly oxidize certain aromatic compounds, many xenobiotics exhibit redox potentials that exceed the oxidative capacity of the T1 copper centre. This limitation led to the development of laccase–mediator systems (LMS), which significantly broaden the catalytic range of laccases. In LMS, the enzyme oxidizes a small, diffusible mediator molecule rather than the target pollutant directly, generating a highly reactive radical form *(Bourbonnais et al., 1990)*. Mediators such as ABTS, HBT, TEMPO, and the plant-derived compound syringaldehyde operate as highly diffusible redox shuttles, bridging the electron-transfer gap between laccase and structurally inaccessible or high-potential substrates, thereby significantly expanding the enzyme's catalytic scope (*Morozova et al., 2007)*. This approach enables the degradation of non-phenolic lignin models, recalcitrant dyes, pesticides and other high redox potential pollutants, establishing laccase-mediator systems as a high component for an advanced bioremediation process.

The catalytic efficiency of laccases is significantly affected by environmental factors, including pH, temperature, oxygen concentration and the presence of metal ions or inhibitors. Fungal laccases generally achieve peak activity in acidic environments, while bacterial laccases are more tolerant of neutral to alkaline conditions and higher temperatures *(Chauhan et al., 2017; Elsayed et al., 2023)*. These functional differences are the result of evolutionary adaptations, determining the appropriateness of specific laccase types for various remediation scenarios. Agricultural soils, which display considerable variation in pH and organic content, can benefit from the synergistic action of diverse laccase types. Additionally, copper ions frequently enhance catalytic by stabilizing the T1 copper site, whereas halides, heavy metals, and certain organic solvents inhibit oxidation reactions (*Morozova et al., 2007*).

Both organic and inorganic inhibitors may significantly reduce laccase activity in soil conditions, which directly affects the efficiency of enzymatic bioremediation. By interfering with electron transfer mechanisms necessary for catalytic activity, inorganic ions including halides and heavy metals can cause partial or whole inhibition of laccases' copper-containing active sites (*Morozova et al., 2007; Baldrian, 2006).* Therefore, high metal concentrations that are frequently present in contaminated agricultural soils can lower enzyme efficiency, especially when free laccases are used. The organic inhibitors, such as certain pesticides, phenolic chemicals, and aromatic xenobiotics, can also reduce laccase activity by competitive inhibition or by altering the structure of the enzyme *(Giardina et al., 2010; Bonnet et al., 2025).*

Thus, when the laccase-based bioremediation techniques is employed, the presence of laccase inhibitors in soil constitute an important element that must be taken into account *(Morozova et al., 2007; Paraschiv et al., 2022).*

Understanding the structure-function relationship of laccases has also paved the way for targeted enzyme engineering. High-redox-potential fungal laccases generally lack a coordinating axial ligand at the T1 copper, increasing their oxidative power, whereas bacterial and low- redox-potential laccases often possess axial methionine, which lowers their redox potential *(Solomon et al., 2014)*. Through site-directed mutagenesis, these structural elements can be modified to fine-tune catalytic properties, enhance thermal or pH stability, or improve the interaction with specific mediators. Advances in heterologous expression, notably in *Pichia pastoris, Escherichia coli*, and plant systems, have facilitated the production of engineered laccases at industrial scales, expanding, of course, their applicability in this area (*Guan et al., 2025).*

In bioremediation, laccases offer mechanistic advantages not only through their oxidation capabilities, but also by acting synergistically with other microbial enzymes and natural soil processes. The catalytic activity of laccases increases the susceptibility of pollutants to subsequent microbial degradation, establishing a cooperative sequence of reactions that accelerates mineralization *(Thathola et al., 2024).* Additionally, since laccase mediated oxidation depends on oxygen, the availability of oxygen in the soil matrix is a key factor influencing the rate and extent of pollutant transformation *(Ezra et al., 2025; Liu et al., 2025).*

In soil environments, laccase-mediated bioremediation occurs predominantly under heterogeneous conditions, where enzymatic reactions take place at the solid–liquid interface rather than in homogeneous solution. Laccases are mainly active as extracellular enzymes and rapidly adsorb onto clay minerals and organic matter through electrostatic and hydrophobic interactions, which can enhance enzyme stability while simultaneously limiting substrate accessibility due to diffusion constraints *(Baldrian, 2006; Morozova et al., 2007).* In this context, the catalytic efficiency of laccases depends strongly on soil texture, mineral composition, and organic matter content.

Because a large fraction of soil contaminants is strongly associated with mineral and organic solid phases, laccase-driven transformations in soils frequently proceed through indirect oxidation pathways involving low-molecular-weight redox mediators. Naturally occurring humic substances and lignin-derived phenolics can act as electron shuttles, allowing oxidative reactions to occur at locations spatially separated from the enzyme itself and enabling the transformation of contaminants bound to soil particles *(Morozova et al., 2007; Paraschiv et al., 2022).* Such solid-phase catalytic processes are especially important for persistent organic pollutants, as the initial laccase-mediated oxidation alters contaminant structure, increases chemical reactivity, and enhances bioavailability, thereby promoting subsequent microbial degradation and mineralization *(Nannipieri et al., 2002; Ezra et al., 2025).*

The catalytic mechanism of laccases, which involves multicopper redox chemistry, long-range electron transfer, and mediator-assisted radical formation, makes these enzymes effective at breaking down persistent pollutants. While the previous section focused on the variety and distribution of microbial laccase producers, this section explains the biochemical features that allow laccases to play a key role in sustainable remediation.

USE OF LACCASES IN THE BIOREMEDIATION OF POLLUTED SOILS

The contamination of soil, water, and air with toxic chemicals has become a major environmental issue as a result of accelerated industrialization, the expansion of intensive agricultural practices, and the widespread use of pesticides. Industrial processes, emissions from energy-related activities, improper waste disposal, and the repeated application of agrochemicals have all contributed to the progressive accumulation of toxic compounds in terrestrial ecosystems. In the United States, approximately 80 billion pounds of hazardous organo- pollutants are produced annually, of which only about 10% are safely eliminated *(Upadhyay et al., 2016; Viswanath et. al., 2014)*. This contamination negatively affects the soil ecosystem and human health, while also reducing crop yields *(Balogu et al., 2017)*.

Agricultural soils are diverse systems where the bioremediation using laccases is mostly affected by soil physicochemical properties. Soil composition, content of organic material, acidity, and mineral content influence enzyme movement, stability, and their interactions with contaminants. Sandy soils usually increase the enzyme diffusion but ensure a minimal protection from inactivation, while clay-heavy soils can limit substrate availability but will improve enzyme stabilization through adsorption. In soils rich in organic matter, humic substances may either block laccase activity or increase pollutant degradation by stabilizing enzymes and supporting redox mediation *(Nannipieri et al., 2002; Morozova et al., 2007; Ezra et al., 2025).*

Soil, often described as a "universal sink," has the capacity to accumulate an extremely diverse range of pollutants *(Sridharan and Krishnaswamy, 2021)*. Persistent pollutants such as PAHs (polycyclic aromatic hydrocarbons), PCP (penta- chloro- phenols), PCBs (polychlorinated biphenyls), DDT (dichloro-diphenyl-trichloroethane), BTEX compounds (benzene, toluene, ethylbenzene, and xylene), and TNT (trinitrotoluene) pose carcinogenic and mutagenic risks, which underscores the need to develop sustainable remediation strategies capable of reducing the environmental impact of pollution and restoring the ecosystem functions of contaminated soils (figure 7) *(Viswanath et. al., 2014)*.

In this context, laccases are distinguished by their ability to oxidize toxic organic pollutants, including polycyclic aromatic hydrocarbons and chlorophenols. An effective strategy for their application in soil is inoculation with laccase-producing fungi, as the use of purified enzymes is not cost-effective on a large scale (*Viswanath et. al., 2014; Upadhyay et al., 2016)*. Enzymes can sequester toxic metals or transform them into less dangerous forms, reducing their bioavailability to plants, and can degrade persistent organic pollutants such as organochlorine pesticides. In the case of petroleum hydrocarbons, laccases disrupt aromatic nuclei, contributing to improved soil fertility and quality. Due to these multiple mechanisms, laccases are considered effective tools for remediating contaminated soils and groundwater *(Hussain et al., 2025)*.

Fig. 7 - Laccases in the bioremediation of polluted soils
(adapted from Karigar et Rao, 2011)

Numerous microorganisms, including bacteria, yeasts, and fungi, are capable of degrading polycyclic aromatic hydrocarbons (PAHs). Bacteria can use PAHs as a carbon source, but they do not mineralize them completely, unlike soil fungi. A promising approach is the use of microbial consortia, combining the advantages of both types of organisms *(Mougin et al., 2023)*. White rot fungi have proven to be particularly effective in degrading PAHs, as they produce extracellular laccase, an enzyme that oxidizes PAHs into quinone-type intermediates and subsequently facilitates their complete mineralization to carbon dioxide *(Mougin et al., 2023; Chandra and Chowdhary, 2015; Liu et al., 2017)*.

The degradation of PAHs can be significantly amplified by the presence of chemical mediators, which are transformed by laccases into reactive species capable of generating oxidative reactions on pollutants. In the presence of these mediators, laccase expands the range of substrates on which it can act, overcoming the limitations imposed by the rigid and hydrophobic structure of PAHs. Through radical mechanisms, the mediators activate chains of reactions that lead to oxidation and subsequently to the opening of aromatic rings, facilitating the mineralization of compounds into less toxic products. Under these optimized conditions, studies have reported oxidation rates of up to 80% for molecules such as anthracene, phenanthrene, or benzo[a]pyrene, highlighting the efficiency of laccase–mediator systems in remediating persistent pollutants. Also, certain natural compounds, such as tyrosine and cysteine, can function as effective mediators in these processes, providing an environmentally friendly alternative to synthetic mediators and contributing to the sustainability of bioremediation strategies *(Mougin et al., 2023)*.

Laccase obtained from the fungi *Trametes versicolor* and *Pleurotus ostreatus* has been successfully used for the degradation of polychlorinated biphenyls as well as phenolic compounds. These fungal enzymes exhibit a high oxidative capacity, which makes them valuable in the bioremediation of persistent organic pollutants and toxic aromatic substances. *(Mishra et al., 2015)*.

Fungal laccases exhibit biotechnological potential for heavy metal decontamination through mechanisms such as chelation, reduction, and precipitation of metal ions. These enzymes can interact with metals via functional groups within their structure, converting toxic forms into less hazardous ones, and can adsorb ions such as Cr(VI), Cu(II), and As(V). However, their efficiency depends on enzyme selectivity, the availability of metal ions, and the concentration of metals in the polluted environment, as high levels of contamination may inhibit enzymatic activity *(Hussain et al., 2025).*

In the study conducted by Deshmukh et al., it was demonstrated that *Aspergillus flavus* and *Aspergillus niger* were capable of reducing heavy metals such as Cr^{6+} to less toxic forms, such as Cr^{3+}. Additionally, the authors of another study *(Mumtaz et al., 2013)* reported that fungi including *Aspergillus*, *Cryptococcus*, *Penicillium*, and *Curvularia* exhibit significant potential in the bioremediation of uranium-contaminated soils due to their ability to bind and immobilize this metal.

Fungal laccases are also capable of degrading various types of pesticides and herbicides, including organophosphates, organochlorines, and carbamates, through the oxidation of aromatic rings, which facilitates their breakdown into less toxic products *(Hussain et al., 2025; Yadav et al. 2025; Younus et al., 2025)*. The resulting products then become more easily accessible to native microbial communities, which complete the mineralization process. The synergistic interaction between laccase and the soil microbiota enhances the natural resilience of contaminated ecosystems, providing a sustainable solution for restoring soil health *(Younus et al., 2025)*. Enzymes have demonstrated effectiveness in degrading several commonly used pesticides, such as atrazine, diazinon, chlorpyrifos, and lindane, through the cleavage of benzene rings. However, the efficiency of laccases varies depending on the type of pesticide, and highly chlorinated molecules are more difficult to degrade *(Hussain et al., 2025)*.

The results of the study conducted by Zhao et al. indicate that laccase produced by white-rot fungi can serve as an effective solution for the remediation of dichloro-diphenyl-trichloroethane (DDT)-contaminated soils, particularly when environmental conditions such as soil aeration and pH are favourable. A high oxygen level enhances the degradation process, after 25 days, DDT residues in oxygenated conditions decreased by 28.1% compared with soils maintained under a nitrogen atmosphere. This approach highlights the potential of enzymes in restoring soil health *(Zhao et Yi, 2010)*.

Species within the genus *Aspergillus* are well known for their versatility in degrading a wide range of toxic compounds, including heavy metals, textile dyes, aromatic compounds, and pesticides *(Deshmukh et al., 2016)*. The species *Trametes versicolor* is used in the bioremediation of soils contaminated with atrazine due to its ability to produce oxidative enzymes capable of degrading this persistent herbicide. The high enzymatic activity of this fungus facilitates the transformation of atrazine into less toxic compounds, making it an effective biological agent in strategies for the remediation of contaminated agricultural soils *(Mishra et al., 2015)*.

The textile, leather, and synthetic dye industries generate massive amounts of effluents each year, many of which reach soils located near industrialized areas. Azo, anthraquinone, and triphenylmethane dyes are known for their persistence in the environment and their high toxicity, exhibiting mutagenic, carcinogenic, and endocrine-disrupting potential. In soil, these molecules form stable associations with organic and mineral fractions, which makes them difficult to remove using conventional physicochemical processes.

In this context, fungal laccases have emerged as a promising alternative for the detoxification of dye-contaminated effluents infiltrated into soils *(Panchal et al., 2024)*. Recent studies have shown that a strain of *Stenotrophomonas maltophilia* synthesizes laccase, an enzyme that was subsequently utilized for the efficient degradation of synthetic dyes *(Chandra and Chowdhary, 2015)*.

Additionally, laccases from fungi such as *Trametes versicolor*, *Pycnoporus cinnabarinus*, and *Cerrena unicolor* are involved in the degradation of a wide range of xenobiotics, from azo dyes to polychlorophenols, due to their ability to oxidize aromatic rings and facilitate subsequent mineralization by soil microorganisms *(Mousavi et al., 2021).*

The paper industry and the biofuel sector make extensive use of laccase due to its high catalytic activity and low substrate specificity. This enzyme can effectively break down a wide range of dyes and aromatic compounds, according to recent research. Owing to this versatility, laccases play a crucial role in the degradation of persistent pollutants and in the transformation of toxic substances into products with reduced or negligible toxicity *(Othman and Flaifil, 2025)*.

The use of enzyme-based bioremediation enables a much more efficient and selective treatment of pollutants, as enzymes exhibit a superior degradation capacity compared with intact microorganisms. Characteristics such as the requirement for mild operating conditions and a low activation energy provide enzymatic bioremediation with high adaptability, making it particularly suitable for applications across diverse environmental settings *(Dong et al., 2023).*

Given the current challenges related to environmental pollution and soil health, enzymatic bioremediation has the potential to serve as a sustainable and relevant solution for restoring soils and protecting both human health and ecosystem integrity.

LIFE CYCLE ASSESSMENT OF LACCASE-BASED BIOREMEDIATION SYSTEMS

The integration of laccase enzymes into bioremediation systems has gained considerable attention in recent years due to the enzymes' broad substrate specificity and eco-friendly mode of action. Life Cycle Assessment (LCA), as a holistic tool, provides an invaluable framework for evaluating the environmental, economic, and operational impacts of such systems throughout their lifespan—from raw material extraction to end-of-life disposal or reuse. Figure 8 illustrates the life cycle assessment of laccase-based bioremediation systems.

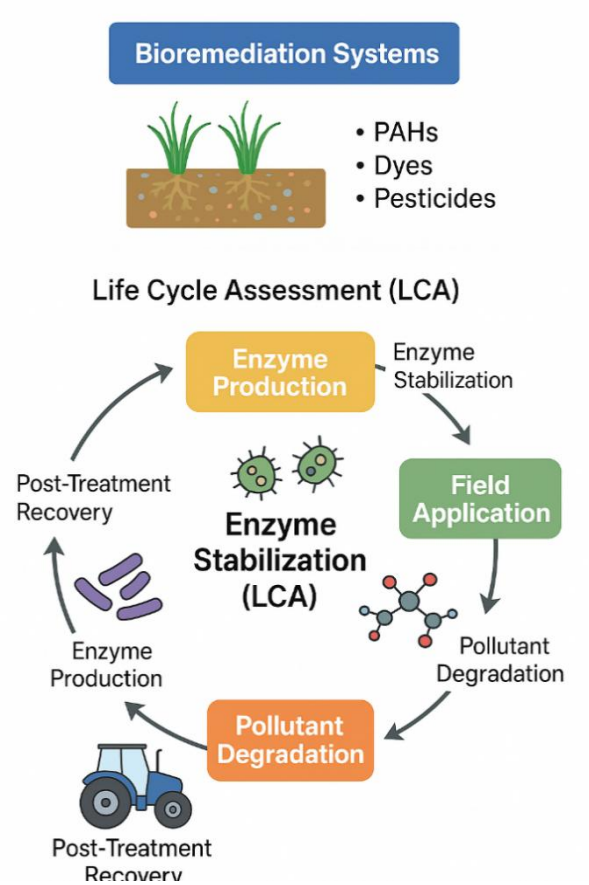

Fig. 8 – LCA and bioremediation systems *(authors own creation – Microsoft Visio)*

Laccase-based bioremediation systems typically involve multiple stages, including enzyme production (through microbial cultivation or recombinant synthesis), enzyme stabilization (via immobilization or hybrid material development), field application, pollutant degradation, and post-treatment soil recovery. Each phase contributes uniquely to the environmental footprint, and its inclusion in the LCA scope is critical to an accurate sustainability assessment.

In order to improve the stability and reusability of laccases in soil environments, several immobilization techniques have been explored. Those techniques can be classified by the type of interaction between the enzyme and the support material. The physical immobilization (like adsorption to soil minerals or organic matter) is the method most employed in soil bioremediation, due to the simplicity and the environmental compatibility. However, partial enzyme desorption and decreased long-term efficacy may result from changes

in soil pH, moisture content, or ionic strength *(Baldrian, 2006; Morozova et al., 2007).* In the category of chemical immobilization can be included various methods, such as: covalent attachment or cross-linking to solid matrices (like chitosan or modified polymers), entrapment or encapsulation of laccases within polymeric or inorganic matrices, hybrid immobilization methods (especially those based on biomineralization or enzyme–inorganic composites). Those immobilization methods generally provide greater resistance to enzymatic deactivation and allow repeated use under variable conditions *(Aslam et al., 2021; Jiang et al., 2018; Hobucsh et al., 2024).*

The goal of an LCA in the context of laccase-based bioremediation is to quantify the environmental impacts over the system's entire life cycle—from enzyme production to application, reuse, and final disposal. The functional unit typically considered is the number of pollutants removed or the volume of soil/water treated. Depending on the study, boundaries may be set from enzyme production or cradle to grave (including disposal and reuse phases) (*Hobucsh et al., 2024).*

According to *(Zhang et al. 2021)* while immobilisation enhances enzyme performance, challenges remain regarding cost-effectiveness, scalable production, and the practicality of synthesis methods. A novel immobilization technique based on biomineralization has recently emerged as a promising strategy for enzyme stabilization (*Ge et al. 2012*). This approach involves a straightforward and mild incubation process that avoids the generation of toxic byproducts. The resulting enzyme–inorganic hybrid nanoflowers demonstrate superior catalytic activity and improved reusability when compared to their free enzyme counterparts (*Jiang et al. 2018; Kumar et al. 2018; Menon et al. 2019, Sun et al. 2020, Wu et al. 2019*). Nevertheless, the application of these hybrid nanoflowers in the biodegradation of azo dyes remains largely unexplored.

Similarly, regarding immobilization strategies, authors (*Aslam et al., 2021*) through their research showed that extracellular laccase derived from *Pleurotus nebrodensis* WC 850 was successfully immobilized onto robust, high-quality spherical chitosan beads to evaluate its potential for the degradation and detoxification of a variety of textile reactive and disperse dyes. The immobilized system (CTS-Lac) demonstrated broad operational stability across diverse pH and temperature ranges and exhibited high degradation efficiencies for all dyes tested. Notably, CTS-Lac retained more than 50% of its initial enzymatic activity toward Foron Turquoise even after eight consecutive dye treatment cycles. The system's strong decolorization performance, notable reusability, and effective reduction in biological oxygen demand (BOD), chemical oxygen demand (COD), and total organic content underscore its significant potential for application in the treatment of dye-laden wastewater and effluents from the textile industry.

Most studies mention the need for proper characterization of the environment and industrial applications in order to be able to apply the proper enzymes. Also, researchers have successfully purified and characterized over 150 fungal laccases, many of which have been explored for a wide range of practical applications (*Darshan et al., 2020*). Further analysis revealed that fungal laccases have been widely employed in the degradation of various environmental pollutants, including lignin, synthetic dyes, phenolic compounds, pesticides, and other hazardous substances. Among these, pesticides are extensively used in both agricultural and domestic settings to control pests. However, their overuse has led to significant environmental contamination and poses risks to human health. As such, effective degradation strategies are essential. Fungal laccases offer a promising solution, as they are capable of breaking down pesticide residues without generating secondary toxic byproducts, making them a more environmentally benign alternative compared to conventional remediation methods (*Darshan et al., 2020*).

The enzyme production phase remains a dominant contributor to environmental impacts, largely due to the energy and nutrient demands of microbial cultivation. An efficient upstream design is, therefore, crucial. Several studies within our dataset highlight attempts to optimize production through selective strain engineering and low-cost substrates. For example, *Zhang et al. (2015)* report on enhanced laccase production using metal-tolerant fungal strains that demonstrate robustness under stress conditions, which can be particularly advantageous in polluted sites with heavy metals (*Zhang et al., 2015*). These advances not only mitigate input costs but also reduce secondary contamination risks.

In terms of application, the performance of laccase in the presence of real-world pollutants such as polycyclic aromatic hydrocarbons (PAHs), azo dyes, and pesticides determines both the duration and effectiveness of bioremediation efforts. The decolorization of industrial dyes, as evidenced in the work of *Li et al. (2020*), underscores the potential for laccase systems to outperform conventional chemical oxidants in specific applications (*Li et al., 2020*). Moreover, hybrid application modes, combining laccase with mild oxidants or redox mediators, are increasingly reported as effective methods for enhancing substrate affinity and reaction kinetics, as noted in *Wang et al., (2021).*

Life Cycle Inventory (LCI) data gathered by scientists suggests key environmental hotspots lie in the energy-intensive purification stages and in the preparation of immobilization matrices. Furthermore, the durability and recyclability of these supports are essential for amortizing environmental costs over extended use cycles. Likewise, the downstream fate of pollutant residues, bioproducts and any immobilization supports must be scrutinized to avoid burden shifting from one environmental compartment to another (*Li et al., 2022*). According to *Wu et al.* (2021), key contributors to environmental burdens include fungal cultivation (*Trametes versicolor, Pleurotus sp*.), enzyme purification, and support material synthesis. For example, one study showed that immobilization using glutaraldehyde and chitosan-coated supports improved enzyme reusability but also introduced chemical residues and synthetic inputs with distinct environmental implications (*Zheng et al., 2017*). A comparison between free and immobilized laccase systems indicated that immobilized systems, despite requiring additional inputs, offset their impacts through prolonged operational lifespan and higher degradation efficiencies. Furthermore, enzyme production from agro-waste substrates such as wheat bran and lignocellulosic residues has been proposed as a sustainable alternative, reducing reliance on energy-intensive growth media (*Financie et al., 2016*).

A key advantage noted across studies is the minimal secondary pollution generated by laccase applications. Unlike conventional chemical oxidants or heavy metal catalysts, laccases degrade pollutants into benign end-products such as CO_2 and water, significantly lowering ecotoxicity indices (*Kandasamy et al., 2016; Gupta et al., 2015*). These factors support their integration into broader sustainability frameworks, particularly in regions with agricultural runoff or persistent organic pollutants.

From a broader perspective, the methodological diversity of LCA applications in laccase research reveals a need for harmonisation. Variations in system boundaries, functional units, and impact categories need cross-study comparability. Nonetheless, works such as those by (*Ezike et al. 2021*), offer frameworks that align LCA with field-scale validations, integrating both environmental indicators and socio-economic considerations. These integrative approaches may be instrumental in positioning laccase-based systems as credible alternatives to energy-intensive or chemically aggressive soil decontamination technologies.

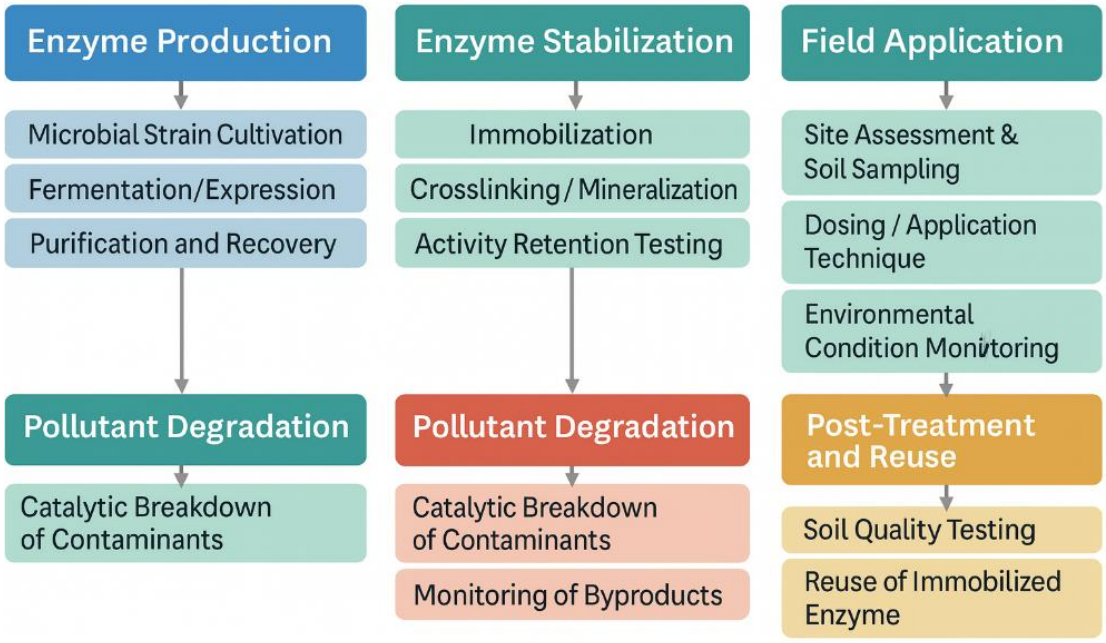

Fig. 9 – LCA Assessment Diagram (authors own creation)

Thus, Life Cycle Assessment of laccase-driven soil bioremediation systems reveals promising environmental credentials, especially when enzyme production and application phases are optimised.

CONCLUSIONS

In recent years, laccases have gained considerable interest due to their oxidative capacity and the essential role they have in the bioremediation processes of contaminated soils, contributing significantly to reducing the impact on the environment.

The selection of a bioremediation method is influenced by a range of factors, including—though not limited to—the cost, the characteristics of the site, and the type and concentration of contaminants present. The specific characteristics of the site contribute to identify the most appropriate and promising bioremediation method (in-situ or ex-situ). Gaining a deeper understanding of how microbial communities in contaminated soils react to specific pollutants will make it easier to identify suitable microorganisms from the affected site for use in bioremediation field tests. This insight will ultimately strengthen the ability to break down the pollutant effectively.

Restoring the quality of soils affected by industrial and agricultural activities requires sustainable solutions, capable of reducing the impact of toxic substances and reactivating natural ecosystem functions. Biological processes represent a promising direction in this regard, as they harness the capacity of organisms and natural enzymes to transform contaminants into less harmful forms and support environmental regeneration. Approaches based on advanced biochemical mechanisms offer selective, efficient and adaptable treatments to a variety of environmental conditions, contributing to maintaining soil health and protecting human health. In the context of increasing pressures on the environment, such biological strategies are emerging as essential tools for responsible resource management and promoting a sustainable development model.

Regarding Life Cycle Assessment, achieving sustainable deployment at scale will require not only technical improvements but also methodological alignment in LCA practices. This would ensure that environmental claims are verifiable, system-level trade-offs are understood, and future innovations are guided by robust, evidence-based sustainability metrics.

ACKNOWLEDGEMENT

This work was supported by a grant from the National Program for Research of the National Association of Technical Universities - GNAC ARUT 2023.

REFERENCES

[1] Abu -Tahon M.A., Housseiny M.M., Aboelmagd H.I., Daifalla N., Khalili M., Isichei A.C., Ramadan A., Abu El-Saad A.M., Seddek N.H., Ebrahim D., Ali Y.H., Saeed I.K., Rikabi H.A., & Eltaib L. (2025). A holistic perspective on the efficiency of microbial enzymes in bioremediation process: Mechanism and challenges: A review. *International Journal of Biological Macromolecules,* 308, 142278. https://doi.org/10.1016/j.ijbiomac.2025.142278

[2] Acién, F., Gómez-Serrano, C., Morales-Amaral, M. M., Sevilla, J. M. F., & Molina-Grima, E. (2016). Wastewater treatment using microalgae: How realistic a contribution might it be to significant urban wastewater treatment? *Applied Microbiology and Biotechnology,* 100, 9013-9022, https://doi.org/10.1007/s00253-016-7835-7

[3] Adams G.O., Fufeyin P.T., Okoro S.E., & Ehinomen I. (2020). Bioremediation, Biostimulation and Bioaugmention: A Review. *International Journal of Environmental Bioremediation & Biodegradation*, 3, 28–39. DOI:10.12691/IJEBB-3-1-5

[4] Alori E.T., Fawole O.B. (2017). Microbial inoculants-assisted phytoremediation for sustainable soil management. In: AA Ansari, R Gill, SS Gill, GR Lanza and L Newman, editors. Phytoremediation: Management of environmental contaminants, vol. 5. Switzerland: Springer International Publishing p. 3–17.

[5] Alori E.T., Gabasawa A. I., Elenwo C. E., & Agbeyegbe O. O. (2022). Bioremediation techniques as affected by limiting factors in soil environment. *Frontiers in Soil Science*, *2*, 937186. https://doi.org/10.3389/fsoil.2022.937186

[6] Anae J., Ahmad N., Kumar V., Thakur V.K., Gutierrez T., Yang X.J., Cai C., Yang Z., Coulon, F. (2021). Recent advances in biochar engineering for soil contaminated with complex chemical mixtures: Remediation strategies and future perspectives. *The Science of the total environment*, *767*, 144351. https://doi.org/10.1016/j.scitotenv.2020.144351

[7] Anekwe I.M.S., & Isa Y.M. (2021). Comparative evaluation of wastewater and bioventing system for the treatment of acid mine drainage contaminated soils. *Water-Energy Nexus*, 4, 134–140. https://doi.org/10.1016/j.wen.2021.08.001.

[8] Anekwe, I.M., & Isa, Y.M. (2021). Wastewater and Bioventing Treatment Systems for Acid Mine Drainage–Contaminated Soil. *Soil and Sediment Contamination: An International Journal, 30*, 518 - 531. DOI:10.1080/15320383.2020.1863909

[9] Aslam S., Asgher M., Khan A.N., Bilal M. (2021). Immobilization of *Pleurotus nebrodensis* WC 850 laccase on glutaraldehyde cross-linked chitosan beads for enhanced biocatalytic degradation of textile dyes. *Journal of Water Process Engineering*, 40, 101971, ISSN 2214-7144, https://doi.org/10.1016/j.jwpe.2021.101971

[10] Azubuike C.C., Chikere C.B., & Okpokwasili G.C. (2016). Bioremediation techniques-classification based on site of application: principles, advantages, limitations and prospects. *World journal of microbiology & biotechnology*, *32*(11), 180. https://doi.org/10.1007/s11274-016-2137-x

[11] Bala S., Garg D., Thirumalesh B.V., Sharma M., Sridhar K., Inbaraj B.S., & Tripathi, M. (2022). Recent Strategies for Bioremediation of Emerging Pollutants: A Review for a Green and Sustainable Environment. *Toxics*, *10*, 484. https://doi.org/10.3390/toxics10080484

[12] Balcazar-Lopez E., Mendez-Lorenzo L.H., Batista-Garcia R.A., Esquivel-Naranjo U., Ayala M., Kumar V.V., Folch-Mallol J.L., & Herrera-Estrella A. (2016). Xenobiotic compounds degradation by heterologous expression of a *Trametes sanguineus* laccase in *Trichoderma atroviride*. *PLoS ONE, 11*(1), e0147997. https://doi.org/10.1371/journal.pone.014799

[13] Baldrian P. (2006). Fungal laccases—Occurrence and properties. *FEMS Microbiology Reviews, 30*, 215–242. https://doi.org/10.1111/j.1574-6976.2005.00010.x

[14] Balogu V.T., MohammedG. R., Mohammed S. D S., Yewodo J. C. (2017). Optimizing Bioremediation of Phenol Polluted Soil Using Laccase from Soil Fungi. *International Journal of Life Sciences Research*, *5*, 62-68. ISSN 2348-3148

[15] Bhatt P., Bhatt K., Chen W.J., Huang Y., Wu S., Lei Q., Zhong J., Zhu X., & Chen S. (2023). Bioremediation potential of laccase for catalysis of glyphosate, isoproturon, lignin, and parathion: Molecular docking, dynamics, and simulation. *Journal of Hazardous Materials*, 443, Part B, 130319. https://doi.org/10.1016/j.jhazmat.2022.130319

[16] Bhelose, D., & Malik, N. (2024). Application of laccase produced by marine actinomycetes in accelerating polyethylene biodegradation. Hrvatska Revija Za Poljoprivrednu Oekologiju, 14(1), 14–23.

[17] Bonnet O., Fa'aui T., Leung I.K.H., Yi S., & Zhuang W.Q. (2025). Chapter 7 - Challenges and applications of laccase in bioremediation. Editor(s): Ivanhoe K.H. Leung, In Foundations and Frontiers in Enzymology, Laccase and Polyphenol Oxidase, *Academic Press*, 153-185. ISBN 9780443133015, https://doi.org/10.1016/B978-0-443-13301-5.00007-X

[18] Bourbonnais, R., & Paice, M. G. (1990). Oxidation of non-phenolic substrates: An expanded role for laccase in lignin biodegradation. FEBS Letters, 267(1), 99–102.

[19] Brugnari T., Braga D.M., dos Santos C.S.A., da Silva E., Baffi M.A., & Peralta, R.M. (2021). Laccases as green and versatile biocatalysts: From lab to enzyme market—an overview. *Bioresources and Bioprocessing, 8*, 131. https://doi.org/10.1186/s40643-021-00484-1

[20] Brijwani, K., Rigdon, A., & Vadlani, P. V. (2010). Fungal laccases: Production, function, and applications. Enzyme Research, 2010, 149748.

[21] Cañas A.I., & Camarero S. (2010). Laccases and their natural mediators: Biotechnological tools for sustainable eco-friendly processes. *Biotechnology Advances, 28*, 694–705. https://doi.org/10.1016/j.biotechadv.2010.05.002

[22] Chandra R., Chowdhary P. (2015). Properties of Bacterial Laccases and Their Application for Bioremediation of Industrial Wastes. *Environmental Science: Processes and Impacts, 17*(2). DOI:10.1039/C4EM00627E

[23] Chandra V., Arpita K., Yadav P., Raghuvanshi V., Yadav A., Ali S., Tripathi V. M., Prajapati S. (2023). Recombinant Laccase: A Promising Tool for Industrial Effluent Bioremediation. *Reports*, *3*(2): 16-22. DOI 10.11648/j.reports.20230302.12

[24] Chauhan, A. (2017). Bacterial laccases: Diversity, characteristics, and biotechnological applications. Frontiers in Microbiology, 8, 1051.

[25] Cui, X., Liu, Y., Cao, C., Xia, Y., Aligayev, Q., Huang, A., & Qing, M. (2024). CoNi–MOF laccase-like nanozymes prepared by dielectric barrier discharge plasma for antibiotic degradation. Environmental Science: Advances.

[26] Cui, X., Liu, Y., Cao, C., Xia, Y., Aligayev, Q., Huang, A., & Qing, M. (2025). CoNi-MOF laccase-like nanozymes prepared by dielectric barrier discharge plasma for treatment of antibiotic pollution. arXiv Preprint.

[27] Davoodi S.M., Miri S., Taheran M., Brar S.K., Galvez-Cloutier R., & Martel R. (2020). Bioremediation of Unconventional Oil Contaminated Ecosystems under Natural and Assisted Conditions: A Review. *Environmental science & technology*, *54*(4), 2054–2067. https://doi.org/10.1021/acs.est.9b00906

[28] Declercq I., Cappuyns V., & Duclos Y. (2012). Monitored natural attenuation (MNA) of contaminated soils: state of the art in Europe--a critical evaluation. *The Science of the total environment*, *426*, 393–405. https://doi.org/10.1016/j.scitotenv.2012.03.040

[29] Deshmukh R., Khardenavis A.A., Purohit H.J. (2016). Diverse Metabolic Capacities of Fungi for Bioremediation. *Indian J Microbiol*. *23*;56(3):247–264. doi: 10.1007/s12088-016-0584-6

[30] Dincă M.N., Ferdes M., Istrate I., Constantin G.A., Paraschiv G., Ionescu M., Zăbavă B.Ș., Musuroi G., Moiceanu G., Rusănescu C. (2025). Bioremediation of soils contaminated with hydrocarbons using microbial consortia. *International Symposium ISB-INMA-TEH - Agricultural and Mechanical Engineering*, 140-147, Bucharest.

[31] Dokua D. (2025). Global pesticide agricultural use 1990-2022. *Statista*. https://www.statista.com/statistics/1263077/global-pesticide-agricultural-use/?srsltid=AfmBOorFfidPuqGMEk5nx19LQF207EWFpQaM-ijb34Y4yW4Uc2c9B19U

[32] Dong C.-D., Tiwari A., Anisha G.S., Chen C.-W., Singh A., Haldar D., Patel A.K., & Singhania, R.R. (2023). Laccase: A potential biocatalyst for pollutant degradation. *Environmental Pollution, 319*, 120999. https://doi.org/10.1016/j.envpol.2023.120999

[33] Ebuehi O., Abibo I., Shekwolo P., Sigismund K., Adoki A., & Okoro I. (2005). Remediation of crude oil contaminated soil by enhanced natural attenuation technique. *Journal of Applied Sciences and Environmental Management*, *9*(1), 103-106. https://doi.org/10.4314/jasem.v9i1.17265

[34] Elsayed, M., Abdelmageed, M., & Abdelmoneim, T. (2023). Bacterial laccases for harsh environment bioremediation. Environmental Technology & Innovation, 30, 102178.

[35] Ezike T.C., Udeh J.O., Joshua P.E., Ezugwu A.L, Isiwu C.V. , Eze S.O.O, Chilaka F. C. (2021). Substrate specificity of a new laccase from Trametes polyzona WRF03, *Heliyon*, Volume 7, Issue 1, e06080, ISSN 2405-8440, https://doi.org/10.1016/j.heliyon.2021.e06080

[36] Ezra, D., Singh, R., & Zhao, K. (2025). Ecological roles of microbial laccases in agricultural soil. Soil Biology & Biochemistry, 179, 108890.

[37] Feng L., Jiang X., Huang Y., Wen D., Fu F., & Fu R. (2021). Petroleum hydrocarbon-contaminated soil bioremediation assisted by isolated bacterial consortium and sophorolipid. *Environmental Pollution*, *273*, 116476, ISSN 0269-7491. https://doi.org/10.1016/j.envpol.2021.116476

[38] Fernandes, T., Silviera, W., Lopes, M. (2014). Laccases from Actinobacteria—What We Have and What to Expect. Advances in Microbiology, 4, 285-296.

[39] Fitri I., Ni'matuzahroh, & Surtiningsih T. (2017). Bioremediation of oil sludge using a type of nitrogen source and the consortium of bacteria with composting method. *AIP Conference Proceedings*. 1854. 020011. https://doi.org/10.1063/1.4985402

[40] Financie R., Moniruzzaman M., Uemura Y. (2016). Enhanced enzymatic delignification of oil palm biomass with ionic liquid pretreatment, *Biochemical Engineering Journal*, 110, 1-7, ISSN 1369-703X, https://doi.org/10.1016/j.bej.2016.02.008

[41] F.A., Youssef D.G., Shahat F.M., & Abd El-Ghany M.N. (2023). Role of Microorganisms in Biodegradation of Pollutants In Ali G.A.M., Makhlouf A.S.H. (eds), Handbook of Biodegradable Materials. *Springer*, *Cham*. 221-260. https://doi.org/10.1007/978-3-031-09710-2_11

[42] Franca R.D.G., Vieira A., Carvalho G., Oehmen A., Pinheiro H.M., Barreto Crespo M.T., & Lourenço N. D. (2020). *Oerskovia paurometabola* can efficiently decolorize azo dye Acid Red 14 and remove its recalcitrant metabolite. *Ecotoxicology and Environmental Safety, 191*, 110007. https://doi.org/10.1016/j.ecoenv.2019.110007

[43] Gałązka A., Jankiewicz U., & Szczepkowski A. (2023). Biochemical characteristics of laccases and their practical application in the removal of xenobiotics from water. *Applied Sciences, 13*, 4394. https://doi.org/10.3390/app13074394

[44] Galhaup C., Goller S., Peterbauer C. K., Strauss J., & Haltrich D. (2002). Characterization of the major laccase isoenzyme from *Trametes pubescens* and regulation of its synthesis by metal ions. *Microbiology, 148*, 2159–2169. https://doi.org/10.1099/00221287-148-7-2159

[45] Garg S.K., Tripathi M., & Srinath T. (2012). Strategies for chromium bioremediation of tannery effluent. *Reviews of environmental contamination and toxicology*, *217*, 75–140. https://doi.org/10.1007/978-1-4614-2329-4_2

[46] Germaine K.J., Byrne J., Liu X., Keohane J., Culhane J., Lally R.D., Kiwanuka S., Ryan D., & Dowling D.N. (2015). Ecopiling: a combined phytoremediation and passive biopiling system for remediating hydrocarbon impacted soils at field scale. *Frontiers in Plant Science.* 5(756). doi: 10.3389/fpls.2014.00756

[47] Ge J., Lei J., Zare R.N. (2012). Protein-inorganic hybrid nanoflowers, *Nat. Nanotechnol*. 7, 428–432

[48] Giardina P., Faraco V., Pezzella C., Piscitelli A., Vanhulle S., & Sannia G. (2010). Laccases: A never-ending story. *Cellular and Molecular Life Sciences, 67*, 369–385. https://doi.org/10.1007/s00018-009-0169-1

[49] Golka K., Kopps S., & Myslak Z.W. (2004). Carcinogenicity of azo colorants: Influence of solubility and bioavailability. *Toxicology Letters, 151*, 203–210. https://doi.org/10.1016/j.toxlet.2003.11.016

[50] Goodwin, C. M. (2010). Screening for laccases from environmental actinomycete isolates. Master's thesis, University of Cape Town.

[51] Guan, X., Li, Y., & Zhou, Z. (2025). Engineered microbial laccases for improved catalytic efficiency. Biotechnology Advances, 68, 108000.

[52] Gupta, V., Garg, S., Capalash, N., Gupta, N., Sharma, P. (2015). Production of thermo-alkali-stable laccase and xylanase by co-culturing of *Bacillus sp*. and *B. halodurans* for biobleaching of kraft pulp and deinking of waste paper. *Bioprocess Biosyst. Eng*. 38, 947–956. https://doi.org/10.1007/s00449-014-1340-0

[53] Gurkok, S. (2021). Important parameters necessary in the bioreactor for the mass production of biosurfactants. In *Green Sustainable Process for Chemical and Environmental Engineering and Science*; Elsevier: Amsterdam, The Netherlands, 347–365. DOI:10.1016/b978-0-12-823380-1.00020-4

[54] Hasan M., Hasan N., Awual R., Islam M., & Shenashen I. (2020). Biodegradable natural carbohydrate polymeric sustainable adsorbents for efficient toxic dye removal from wastewater. *Journal of Molecular Liquids, 319*, 114356. https://doi.org/10.1016/j.molliq.2020.114356

[55] Hildén K., Hakala T.K., & Lundell T. (2009). Thermotolerant and thermostable laccases. *Biotechnology Letters, 31*, 1117–1128. https://doi.org/10.1007/s10529-009-9995-5

[56] Hilgers R., Vincken J.P., Gruppen H., & Kabel M. A. (2018). Laccase/mediator systems: Their reactivity toward phenolic lignin structures. *ACS Sustainable Chemistry & Engineering*, 6(2), 2037–2046. https://doi.org/10.1021/acssuschemeng.7b03451

[57] Hobusch M., Kırtel O., Meramo S., Sukumara S., Welner D.H. (2024). A life cycle assessment of early-stage enzyme manufacturing simulations from sustainable feedstocks, *Bioresource Technology*, 400, 130653, ISSN 0960-8524, https://doi.org/10.1016/j.biortech.2024.130653

[58] Hussain A., Siddiqui M., Rehman U., Hussain N., Khan N. A., Soomro H. S. P., Abbas M. (2025). Applications of Fungal Laccases in the Bioremediation of Industrial Pollutants. *African Journal of Biological Sciences, 7*(6):196–221. DOI:10.48047/AFJBS.7.6.2025.196-221

[59] Iark D., dos Reis Buzzo A.J., Garcia J.A.A., Correa V.G., Helm C.V., Correa R.C.G., Peralta R.A., Regina de Fatima P., Bracht A., & Peralta R.M. (2019). Enzymatic degradation and detoxification of azo dye Congo red by a new laccase from *Oudemansiella canarii*. *Bioresource Technology, 289*, 121655.

[60] Janssen D.B., & Stucki G. (2020). Perspectives of genetically engineered microbes for groundwater bioremediation. Environmental Science: Processes & Impacts, 22, 487–499. https://doi.org/10.1039/C9EM00601J

[61] Janusz, G., Pawlik, A., Sulej, J., Jarosz-Wilkołaska, A., & Paszczyński, A. (2020). Agricultural soil pollutants and microbial laccase producers for bioremediation. International Biodeterioration & Biodegradation, 152, 104974.

[62] Janusz G., Pawlik A., Świderska-Burek U., Polak J., Sulej J., Jarosz-Wilkołazka A., & Paszczyński A. (2020). Laccase properties, physiological functions, and evolution. *International Journal of Molecular Sciences, 21*, 966. https://doi.org/10.3390/ijms21030966

[63] Jiang W., Wang X., Yang J., Han H., Li Q., Tang T. (2018). Lipase-inorganic hybrid nanoflower constructed through biomimetic mineralization: a new support for biodiesel synthesis, *J. Colloid Interface Sci*. 514, 102–107

[64] Jones S.M., & Solomon E.I. (2015). Electron transfer and reaction mechanism of laccases. *Cellular and Molecular Life Sciences,* 72(5), 869–883. https://doi.org/10.1007/s00018-014-1826-6

[65] Karigar C. S., Rao S. S. (2011). Role of Microbial Enzymes in the Bioremediation of Pollutants: A Review. *Enzyme Research*, Article ID 805187, 11 pages. doi:10.4061/2011/80518

[66] Kandasamy, S., Muniraj, I.K., Purushothaman, N., Sekar, A., Sharmila, D.J.S., Kumarasamy, R., Uthandi, S. (2016). High level secretion of Laccase (LccH) from a newly isolated white-rot Basidiomycete, *Hexagonia hirta MSF2. Front. Microbiol*. 7, 707. https://doi.org/10.3389/fmicb.2016.00707

[67] Kashefi S., Borghei S.M., & Mahmoodi N.M. (2019). Covalently immobilized laccase onto graphene oxide nanosheets: Preparation, characterization, and biodegradation of azo dyes in colored wastewater. *Journal of Molecular Liquids, 276*, 153–162. https://doi.org/10.1016/j.molliq.2019.01.047

[68] Khalid F., Hashmi M.Z., Jamil N., Qadir A., & Ali M.I. (2021). Microbial and enzymatic degradation of PCBs from e-waste-contaminated sites: a review. *Environmental science and pollution research international*, *28*(9), 10474–10487. https://doi.org/10.1007/s11356-020-11996-2

[69] Koshlaf E., & Ball A.S. (2017). Soil bioremediation approaches for petroleum hydrocarbon polluted environments. *AIMS Microbiology*, *3*(1), 25-49, doi: 10.3934/microbiol.2017.1.25

[70] Kües U. (2015). Fungal enzymes for environmental management. *Current Opinion in Biotechnology, 33*, 268–278. https://doi.org/10.1016/j.copbio.2015.03.006

[71] Kumar A., Patel S.K.S. , Mardan B., Pagolu R., Lestari R., Jeong S.-H. , Kim T., Haw J. R. , Kim S.-Y., Kim I.-W., Lee J.-K. (2018) Immobilization of xylanase using a protein-inorganic hybrid system, *J. Microbiol. Biotechnol.* 28 pages, 638–644.

[72] Lee H., Sam K., Coulon F., De Gisi S., Notarnicola M., & Labianca C. (2024). Recent developments and prospects of sustainable remediation treatments for major contaminants in soil: A review. *Science of the Total Environment*, 912, 168769. https://doi.org/10.1016/j.scitotenv.2023.168769

[73] Liu B., Liu J., Ju M., Li X., Wang P. (2017). Bacteria-white-rot fungi joint remediation of petroleum-contaminated soil based on sustained-release of laccase. *RSC Adv.*,*7*, 39075-39081. https://doi.org/10.1039/C7RA06962F

[74] Li, D.C., Xu, W.F., Mu, Y., Yu, H.Q., Jiang, H., Crittenden, J.C. (2018). Remediation of Petroleum-Contaminated Soil and Simultaneous Recovery of Oil by Fast Pyrolysis. *Environmental science & technology*, *52*(9), 5330–5338. https://doi.org/10.1021/acs.est.7b03899

[75] Li M., Karboune S., Light K., & Kermasha S. (2021). Oxidative cross-linking of potato proteins by fungal laccases: Reaction kinetics and effects on the structural and functional properties. *Innovative Food Science and Emerging Technologies, 71*, 102723. https://doi.org/10.1016/j.ifset.2021.102723

[76] Li, Z., Chen, Z., Zhu, Q., Song, J., Li, S., Liu, X. (2020). Improved performance of immobilized laccase on Fe3O4@C-Cu2+ nanoparticles and its application for biodegradation of dyes. *J. Hazard Mater*. 399 https://doi.org/10.1016/j. jhazmat.2020.123088

[77] Li S., Hong D., Chen W., Wang J., Sun K. (2022). Extracellular laccase-activated humification of phenolic pollutants and its application in plant growth, *Science of The Total Environment*, Volume 802, 150005, ISSN 0048-9697, https://doi.org/10.1016/j.scitotenv.2021.150005

[78] Liu, C., Xia, Y., Aligayev, Q., Huang, A. & Qing. (2025). CoNi-MOF laccase-like nanozymes prepared by dielectric barrier discharge plasma for treatment of antibiotic pollution. https://arxiv.org/abs/2510.15524.

[79] Llorca M., Rodríguez-Mozaz S., Couillerot O., Panigoni K., de Gunzburg J., Bayer S., España S., Paredes, J., & Barceló D. (2015). Identification of new transformation products during enzymatic treatment of tetracycline and erythromycin antibiotics at laboratory scale by an on-line turbulent flow-liquid-chromatography coupled to a high resolution mass spectrometer LTQ-Orbitrap. *Chemosphere, 119*, 90–98. https://doi.org/10.1016/j.chemosphere.2014.05.072

[80] Loi M., Glazunova O., Fedorova T., Logrieco A.F., & Mulè G. (2021). Fungal laccases: The forefront of enzymes for sustainability. *Journal of Fungi, 7*, 1048. https://doi.org/10.3390/jof712104

[81] Lopes P.R.M., Cruz V.H., de Menezes A.B., Gadanhoto B.P., Moreira B.R.D.A., Mendes C.R., Mazzeo D.E.C., Dilarri G., & Montagnolli R.N. (2022). Microbial bioremediation of pesticides in agricultural soils: an integrative review on natural attenuation, bioaugmentation and biostimulation. *Reviews in Environmental Science and Biotechnology*, *21*(4), 851-876. https://doi.org/10.1007/s11157-022-09637-w

[82] Lv, Y., Bao, J., Zhu, L. (2022). A comprehensive review of recent and perspective technologies and challenges for the remediation of oil-contaminated sites, *Energy Reports*, 8, 7976-7988, ISSN 2352-4847, https://doi.org/10.1016/j.egyr.2022.06.034

[83] Madhavi V., & Lele S.S. (2009). Laccase: Properties and applications. *BioResources, 4*, 1694–1717
[84] Maiti P., Siddiqi H., Kumari U., Chatterjee A., & Meikap B.C. (2023). Adsorptive remediation of azo dye contaminated wastewater by ZnCl2 modified bio-adsorbent: Batch study and life cycle assessment. *Powder Technology, 415*, 118153. https://doi.org/10.1016/j.powtec.2022.118153
[85] Mahuri, S., Singh, P., & Verma, V. (2023). Microbial laccases: Functional diversity and biotechnological potential. Frontiers in Microbiology, 14, 1145678.
[86] Manhivi V.E., Amonsou E.O., & Kudanga T. (2018). Laccase-mediated crosslinking of gluten-free amadumbe flour improves rheological properties. *Food Chemistry, 264*, 157–163. https://doi.org/10.1016/j.foodchem.2018.05.017
[87] Martinkova L., Kotik M., Markova E., & Homolka, L. (2016). Biodegradation of phenolic compounds by Basidiomycota and its phenol oxidases: A review. *Chemosphere, 149*, 373–382. https://doi.org/10.1016/j.chemosphere.2016.01.022
[88] Mate D.M., & Alcalde M. (2015). Laccase engineering: From rational design to directed evolution. *Biotechnology Advances,* 33, 25-40. https://doi.org/10.1016/j.biotechadv.2014.12.007
[89] Memon A.H., Ding R., Yuan Q., Wei Y., Liang H. (2019). Facile synthesis of alcalase-inorganic hybrid nanoflowers used for soy protein isolate hydrolysis to improve its functional properties, *Food Chem.* 289 pages 568–574.
[90] Micle V., & Sur I. M. (2021). Experimental Investigation of a Pilot-Scale Concerning Ex-Situ Bioremediation of Petroleum Hydrocarbons Contaminated Soils. *Sustainability*, *13*(15), 8165. https://doi.org/10.3390/su13158165
[91] Misal S.A., & Gawai K.R. (2018). Azoreductase: A key player of xenobiotic metabolism. *Bioresources and Bioprocessing, 5*, 17. https://doi.org/10.1186/s40643-018-0206-8
[92] Mishra K. S., Srivastava S. K., Ash K. (2015). Laccase sources and their applications in environmental pollution. *International Journal of Life-Sciences Scientific Research (IJLSSR)*,*1* (2), 71-73
[93] Moreno A.D., Ibarra D., Mialon A., & Ballesteros M. (2016). A bacterial laccase for enhancing saccharification and ethanol fermentation of steam-pretreated biomass. *Fermentation, 2*, 11. https://doi.org/10.3390/fermentation2040011
[94] Morozova, O. V., Shumakovich, G. P., Shleev, S. V., & Yaropolov, A. I. (2007). Laccase–mediator systems and their applications: A review. Applied Biochemistry and Microbiology, 43(5), 523–535.
[95] Mousavi S. M., Hashemi S. A., Moezzi S. M. I., Ravan N., Gholami A., Lai C. W., Chiang W-H., Omidifar N., Yousefi K., Behbudi G. (2021). Recent Advances in Enzymes for the Bioremediation of Pollutants. *Biochem Res Int*., *22*:5599204. doi: 10.1155/2021/5599204
[96] Mumtaz S., Streten-Joyce C., Parry D.L., Mc Guinness K.A., Lu P., Gibb K.S. (2013). Fungi outcompete bacteria under increased uranium concentration in culture media. *J Environ Radioact*., *120*, 39–44. doi: 10.1016/j.jenvrad.2013.01.007
[97] Muniraj, I., Shameer, S., & Uthandi, S. (2022). Tyrosinase and laccase-producing Bacillus aryabhattai TFG5 and its role in polymerization of phenols. BMC Microbiology, 22.
[98] Myazin V.A., Korneykova M.V., Chaporgina A.A., Fokina N.V., & Vasilyeva G.K. (2021). The Effectiveness of Biostimulation, Bioaugmentation and Sorption-Biological Treatment of Soil Contaminated with Petroleum Products in the Russian Subarctic. *Microorganisms*, *9*(8), 1722. https://doi.org/10.3390/microorganisms9081722
[99] Nannipieri P., Kandeler E., & Ruggiero P. (2002). Enzyme activities and microbiological and biochemical processes in soil. In *Enzymes in the Environment*; CRC Press: Boca Raton, FL, USA, 1–33. DOI:10.1201/9780203904039-1
[100] Nuruzzaman M., Mezbaul Bahar M., & Naidu R. (2025). Diffuse soil pollution from agriculture: Impacts and remediation. *Science of The Total Environment,* 962, 178398. https://doi.org/10.1016/j.scitotenv.2025.178398
[101] Ojha, N., Karn, R., Abbas, S., & Bhugra, S. (2021). Bioremediation of Industrial Wastewater: A Review. *IOP Conference Series: Earth and Environmental Science, 796*(1), 012012. doi: 10.1088/1755-1315/796/1/012012
[102] Oprea I., Badea A., Ziglio G., Ragazzi M., Andreottola G., Ferrarese E., & Apostol T. (2009). The remediation of contaminated sediments by chemical oxidation. *U.P.B. Sci. Bull., Series C*, 71, 131-142.
[103] Othman A. M., Flaifil A. G. (2025). Characterization and evaluation of the immobilized laccase enzyme potential in dye degradation via one factor and response surface methodology approaches. *Scientific Reports*, *15*, Article number: 735.

[104] Papageorgiou, A., Azzi, E. S., Enell, A., & Sundberg, C. (2021). Biochar produced from wood waste for soil remediation in Sweden: Carbon sequestration and other environmental impacts. *The Science of the total environment*, *776*, 145953. https://doi.org/10.1016/j.scitotenv.2021.145953

[105] Paraschiv G., Ferdes M., Ionescu M., Moiceanu G., Zabava B.S., & Dinca M.N. (2022). Laccases—Versatile enzymes used to reduce environmental pollution. *Energies, 15*, 1835. https://doi.org/10.3390/en15051835

[106] Patel A.K., Singhania R.R., Albarico F.P.J.B., Pandey A., Chen C.W., & Dong C.D. (2022). Organic wastes bioremediation and its changing prospects. *The Science of the total environment*, *824*, 153889. https://doi.org/10.1016/j.scitotenv.2022.153889

[107] Panchal K., Gera R., Garg R., Kumar R. (2024). Laccase Enzyme: A Sustainable Catalyst For Bioremediation Strategies. *Biosciences Biotechnology Research Asia*. *21*(4):1277-1287. http://dx.doi.org/10.13005/bbra/3303

[108] Pereira-Silva G., Leonardo-Silva L., Camilo-Cotrim C.F., de Araujo Toschi L., Jeronimo dos Reis M., & Xavier-Santos S. (2025). Barriers and guidelines in the use of fungi in pesticide bioremediation: A global overview. *Science of the Total Environment*, 991, 179913. https://doi.org/10.1016/j.scitotenv.2025.179913

[109] Pierrat E., Laurent A., Dorber M., Rygaard M., Verones F., & Hauschild M. (2023). Advancing water footprint assessments: Combining the impacts of water pollution and scarcity. *Science of the Total Environment, 870*, 161910. https://doi.org/10.1016/j.scitotenv.2023.161910

[110] Rodriguez-Rodriguez C.E., Garcia-Galan M.A., Blanquez P., Diaz-Cruz M.S., Barcelo D., & Caminal G. (2012). Continuous degradation of a mixture of sulfonamides by *Trametes versicolor* and identification of metabolites from sulfapyridine and sulfathiazole. *Journal of Hazardous Materials, 213–214*, 347–354. https://doi.org/10.1016/j.jhazmat.2012.02.008

[111] Romantschuk, M., Lahti-Leikas, K., Kontro, M., Galitskaya, P., Talvenmäki, H., Simpanen, S., Allen, J. A., & Sinkkonen, A. (2023). Bioremediation of contaminated soil and groundwater by *in situ* biostimulation. *Frontiers in microbiology*, *14*, 1258148. https://doi.org/10.3389/fmicb.2023.1258148

[112] Romero-Martínez D., Parra-Saldivar R., Trujillo-Roldán M.A., & Valdez-Cruz N.A. (2024). Chapter 7 - Emerging contaminants and their possible bioremediation through bacterial laccases. Editor(s): Deepti Yadav, Tukayi Kudanga, In Progress in Biochemistry and Biotechnology, Bacterial Laccases, *Academic Press*, 141-172. ISBN 9780323918893. https://doi.org/10.1016/B978-0-323-91889-3.00008-X

[113] Rudakiya D.M., Patel D.H., Gupte A. (2020). Exploiting the potential of metal and solvent tolerant laccase from Tricholoma giganteum AGDR1 for the removal of pesticides, International *Journal of Biological Macromolecules*, Volume 144, Pages 586-595, ISSN 0141-8130, https://doi.org/10.1016/j.ijbiomac.2019.12.068

[114] Sales da Silva I.G., Gomes de Almeida F.C., Padilha da Rocha e Silva N.M., Casazza A.A., Converti A., & Asfora Sarubbo L. (2020). Soil Bioremediation: Overview of Technologies and Trends. *Energies*, *13*(18), 4664. https://doi.org/10.3390/en13184664

[115] Santos, M., Rebola, S., Evtuguin, D.V. (2025). Soil Remediation: Current Approaches and Emerging Bio-Based Trends. *Soil Systems, 9*, 35. https://doi.org/10.3390/soilsystems9020035

[116] Schroyen M., Vervaeren H., Van Hulle S.W.H., & Raes K. (2014). Impact of enzymatic pretreatment on corn stover degradation and biogas production. *Bioresource Technology, 173*, 59–66. https://doi.org/10.1016/j.biortech.2014.09.061

[117] Sharma, J. (2019). Advantages and limitations of *in situ* methods of bioremediation. Recent Advances in Biology and Medicine, 5, 10941

[118] Shraddha, Shekher R., Sehgal S., Kamthania M., & Kumar A. (2011). Laccase: microbial sources, production, purification, and potential biotechnological applications. *Enzyme Res.* 217861. doi: 10.4061/2011/217861.

[119] Shraddha, R., Shekher, R., Sehgal, S., Kamthania, M., & Kumar, A. (2011). Laccase-producing microorganisms and their ecological roles. Journal of Environmental Sciences, 23(7), 1185–1196.

[120] Solomon, E. I., Augustine, A. J., & Yoon, J. (2008). O_2 reduction to water by multicopper oxidases. Dalton Transactions, 3921–3932.

[121] Sridharan R. and Krishnaswamy V.G. (2021). Laccases for Soil Bioremediation: An Introduction. In Handbook of Research on Microbial Remediation and Microbial Biotechnology for Sustainable Soil. DOI: 10.4018/978-1-7998-7062-3.ch023

[122] Su C., Jiang LQ., & Zhang WJ. (2014). A review on heavy metal contamination in the soil worldwide: situation, impact and remediation techniques. *Environmental Skeptics and Critics*, 3, 24-38.
[123] Sun X., Niu H., Song J., Jiang D., Leng J., Zhuang W., Chen Y., Liu D., Ying H. (2020). Preparation of a copper polyphosphate kinase hybrid nanoflower and its application in ADP regeneration from AMP, *ACS Omega* 5, 9991–9998.
[124] Thangaraj, S., Sadasivam, S., & Kumar, S. (2020). Dye degradation potential and degradative enzymes of *Bacillus cereus* SKB12 isolated from textile effluent. *Journal of Applied Biology & Biotechnology*, 8.
[125] Uloaku M.I., Abbey S.J., Ifelebuegu A.O. (2022). A systematic review on the effectiveness of remediation methods for oil contaminated soils, *Environmental Advances*, Volume 9, 100319, 657, https://doi.org/10.1016/j.envadv.2022.100319
[126] Upadhyay P., Shrivastava R., Agrawal P. K. (2016). Bioprospecting and biotechnological applications of fungal laccase. *Biotech*., *6*(1):15. doi: 10.1007/s13205-015-0316-3
[127] Vanathi, P., Vimalraj, S., & Radhakrishnan, M. (2024). Actinomycete laccases for degradation of synthetic dyes and plastics. Journal of Environmental Management, 323, 116244.
[128] Viswanath B., Rajesh B., Janardhan A., Kumar A. P., Narasimha G. (2014). Fungal Laccases and Their Applications in Bioremediation. *Enzyme Res*., *15*: 163242. doi: 10.1155/2014/163242
[129] Wang, F., Xu, L., Zhao, L., Ding, Z., Ma, H., & Terry, N. (2019). Solid-state fermentation for enhanced fungal laccase production using agricultural waste. Bioresource Technology, 292, 121934.
[130] Wang L., Rinklebe J., Tack F.M., & Hou D. (2021). A review of green remediation strategies for heavy metal contaminated soil. Soil Use and Management, 37(4), 936–963. https://doi.org/10.1111/sum.12717
[131] Wang, T. , Lu, L. , Li, G. , Li, J. , Xu, T. , Zhao, M. (2011). Decolorization of the azo dye reactive black 5 using laccase mediator system. African *Journal of Biotechnology* 10 (75), 17186–17191
[132] Wu, Y., Jing, X., Gao, C., Huang, Q., & Cai, P. (2018). Recent advances in microbial electrochemical system for soil bioremediation. *Chemosphere*, *211*, 156–163. https://doi.org/10.1016/j.chemosphere.2018.07.089
[133] Wu K., Zhang Y., Sun Q., Chai Y., He Q., Zhou X., He X., Ji H. (2019). Preparation of cytochrome P450 enzyme-cobalt phosphate hybrid nano-flowers for oxidative coupling of benzylamine. *Enzym. Microb. Technol.* 131, 109386
[134] Yadav S., Kundu N., Bhattacharya A., Aseri G.K., Jain N. (2025). Biocatalysts for Change: Microbial Enzymes as a Sustainable Solution to Industrial Pollution. *J Pure Appl Microbiol. 19*(3):1627-1647. https://doi.org/10.22207/JPAM.19.3.34
[135] Yang, X., Li, W., Ng, T., Deng, X., & Lin, J. (2017). High-yield fungal laccase producers: *Trametes versicolor, Pleurotus ostreatus, Cerrena unicolor, Phanerochaete chrysosporium*, and *Lentinus tigrinus*. Bioresource Technology, 245, 1021–1031.
[136] Yesilada O., Birhanli E., & Geckil H. (2018). Bioremediation and decolorization of textile dyes by white rot fungi and laccase enzymes. In R. Prasad (Ed.), *Mycoremediation and Environmental Sustainability* (Vol. 2, pp. 121–153). Springer. https://doi.org/10.1007/978-3-319-77386-5_6
[137] Younus H., Khan M. A., Khan A., Alhumaydhi F. A. (2025). Eco-Friendly Biocatalysts: Laccase Applications, Innovations, and Future Directions in Environmental Remediation. *Catalysts*, *15*(10), 921. https://doi.org/10.3390/catal15100921
[138] Zhang C., You S., Zhang J., Qi W., Su R., & He Z. (2020). An effective in-situ method for laccase immobilization: Excellent activity, effective antibiotic removal rate and low potential ecological risk for degradation products. *Bioresource Technology, 308*, 123271.
[139] Zhang M., Zhang Y., Yang C., Ma C., Zhang Y., Tang J. (2021). Facile synthesis of recyclable laccase-mineral hybrid complexes with enhanced activity and stability for biodegradation of Evans Blue dye. *International Journal of Biological Macromolecules*, 188, 783-789, ISSN 0141-8130,https://doi.org/10.1016/j.ijbiomac.2021.08.058
[140] Zhang J., Chen H., Chen M., Ren A., Huang J., Wang H., Zhao M., Feng Z. (2015). Cloning and functional analysis of a laccase gene during fruiting body formation in Hypsizygus marmoreus, *Microbiological Research*, 179, 54-63, ISSN 0944-5013, https://doi.org/10.1016/j.micres.2015.06.005
[141] Zheng F., An Q., Meng G., Wu X., Dai Y., Si J., Cui B. (2017). A novel laccase from white-rot fungus *Trametes orientalis*: purification, characterisation, and application, *Int. J. Biol. Macromol*. 102, 758–770
[142] Zhao Y., Yi X. (2010). Effects of Soil Oxygen Conditions and Soil pH on Remediation of DDT-contaminated Soil by Laccase from White Rot Fungi. *Int. J. Environ. Res. Public Health*, *7*(4), 1612-1621. https://doi.org/10.3390/ijerph7041612

[143] ***Mougin C., Boukcim H., Jolivalt C. (2023). Soil bioremediation strategies based on the use of fungal enzymes. https://hal.inrae.fr/hal-02824502v1/document (accessed on 20 November 2025).
[144] *** United Nations. https://sdgs.un.org/goals (accessed on 28 November 2025).
[145] ***The Food and Agriculture Organization (FAO) and United Nations Environment Programme (UNEP). (2021). *Global assessment of soil pollution: Report*. Rome. https://openknowledge.fao.org/server/api/core/bitstreams/fe5df8d6-6b19-4def-bdc6-62886d824574/content/src/html/chapter-13-4.html (accessed on 6 November 2025)
[146] ***The Food and Agriculture Organization (FAO), Sources of soil pollution. Available online: https://openknowledge.fao.org/server/api/core/bitstreams/fe5df8d6-6b19-4def-bdc6-62886d824574/content/src/html/chapter-03-3.html (accessed on 27 November 2025).