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Review

Biochemical Characteristics of Laccases and their Practical Application in the Removal of Xenobiotics from Waters

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Abstract: Industrialization, intensive farming, rapid population growth and urbanization are the source of a large number of pollutants entering the environment. The current concentration of xenobiotics released into the environment exceeds its natural ability to decompose them. Enzymatic degradation of pollutants seems to be an environmentally friendly process. Due to the wide spectrum of substrate specificity, from inorganic compounds to high molecular weight organic compounds such as PAH or dyes, as well as favorable biochemical properties, laccase has been used in the biological removal of xenobiotics from the environment. It is important to understand the degradation mechanisms of pollutants and to evaluate the final products in terms of their toxicity. The laccase oxidizes the substrates with the simultaneous reduction of molecular oxygen to water, which is the purest reaction co-substrate. That is why it is called a green biocatalyst. The trend is an increase in the production of enzymes related to the intensive development of industry, bioremediation or synthetic chemistry. This leads to the search for laccases with greater activity and stability under extreme conditions. The potential of laccases to degrade xenobiotics can be promoted by improving enzymatic catalytic characterization using protein engineering and other genetic engineering methods.

Keywords: laccase; xenobiotics; protein engineering

1. Introduction

Laccases are detected in successive groups of organisms. In addition to fungi and bacteria, this enzyme has been found in plants, insects, sponges and lichens [1–5]. Depending on the species, laccases are naturally involved in synthesis or degradation processes. Bifunctional actions (ie catabolic or anabolic) involving laccase-catalysed oxidation of small organic compounds are ubiquitous in in vivo laccase-driven metabolic pathways and contribute to a variety of functions.[6]. In reaction catalysis by laccases of different origin various products of substrate degradation as a result [7].

The quality of water resources has deteriorated significantly in recent years. This is related to the rapid growth of the population, and thus the dynamic industrial development and the intensification of agricultural activity. In order to meet the nutritional needs of people, the use of chemical plant protection products and pharmaceuticals in animal husbandry is increasing in agriculture. As a result of urbanization, intensive agricultural and industrial activities, many xenobiotics, such as pesticides, steroid hormones, antibiotics and dyes, enter the aquatic environment [8,9].

The wide use of dyes is associated with their mass production, which results in the fact that a significant part of them penetrates both water and soil ecosystems. Annually, the textile industry alone generates nearly 200 billion liters of colored wastewater [8,10]. Up to 50% of the amount of industrial wastewater containing dyes is discharged directly into aquatic ecosystems. Azo dyes account for over 70% of global industrial demand (~9 million tonnes) [11].

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Xenobiotics were found also in the drinking water. PAHs are also found in drinking water at concentrations ranging from 1.33 ng /L (for BaP in treated drinking water in Tehran, Iran) to 139,000 ng /L (for PHE in untreated drinking water in Lagos, Nigeria) [12].

Another important group of xenobiotics are pharmaceuticals, which include about 10,000 different pharmaceuticals containing about 3,000-4,000 different active ingredients. Hundreds of tons of pharmaceutical compounds are released and consumed worldwide every year [13]. It has been proven that pharmaceuticals can cause endocrine disorders, change the structure and key functions of natural microbial communities, negatively affect invertebrates and fish, and in the case of antibiotics lead to the development of antibiotic resistant genes and bacteria [13,14]. A significant proportion of PhAC is released into the environment intact or metabolized [15]. The occurrence of pharmaceuticals and their metabolites in surface waters, groundwater, coastal sea waters, drinking water and sediments has been reported [9].

Biodegradation of xenobiotics is most often a multi-stage process and consists in the decomposition of organic substances by microorganisms and enzymes produced by them. The scope and speed of biodegradation changes are conditioned by a number of factors, among which the most important are considered to be: the availability of xenobiotic, the metabolic and degradation potential of the microorganism, oxygenation conditions and the presence of easily assimilable energy and building substrates in the environment [16].

Enzymes exist in every living cell, so they are present in all microbes. The relative amounts of the various enzymes produced by microbes vary from species to species and even between strains of microbes. Enzymes are very specific in their action on substrates, so different enzymes can help degrade specific substances [17]. Laccases are green catalytic enzymes with a high potential for biodegradation of environmental xenobiotics. Laccases are currently being intensively researched for potential use in the biodegradation of xenobiotic compounds, such as pharmaceuticals, dyes or plasticizers [18].

The costs of industrial production of enzymes and their sensitivity to unfavorable conditions, such as temperature, pH or the presence of inhibitors in wastewater, are a problem Optimization of the production of laccases resistant to extreme environmental conditions, immobilization and recombination techniques will in the future, reducing the costs of bioremediation with enzymes [19].

In this review, the biochemical characteristics of laccases of various origins, their functions in nature, potential applications in industry and water bioremediation will be discussed. The mechanisms of laccase-assisted removing xenobiotics from the environment and the possibilities of improving their biochemical properties and productivity by traditional methods and with the use of genetic engineering and protein engineering will also be discussed in general.

2. Characteristics of laccases

The metal-dependent lacases enzymes of the oxidoreductase class [EC 1.10.3.2] are included in the ligninolytic complex. Together with ascorbate oxidases, ceruloplasmin, bilirubin oxidase, phenoxazinone synthase and metallo-oxidase Fet3p, they belong to the Multicopper oxidases (MCOs) family. These enzymes are characterised by their broad substrate specificity and, in the catalysed reaction, they use molecular oxygen as an electron acceptor and produce a water molecule as a result of oxygen reduction. Oxidases of the MCOs type oxidise many polyphenolic substrates and momomeric phenolic compounds, as well as diamines and metal ions [20]. A common feature of the enzymes belonging to the MCO family is a catalytic centre made up of a minimum of four copper atoms divided, due to their spectroscopic and magnetic properties, into three types: T1, T2 and two T3 types. The final product formation occurs by oxidation of the substrate molecules to oxide radicals with simultaneous reduction of an oxygen molecule to two water molecules according to the general notation: $4RH + O2 \rightarrow 4R- + 2H2O$ [21–23]. Lacases form multimetric complexes that can be mono-, di-, or tetrameric glycoproteins. Each monomer has four copper atoms in its catalytic centre: a type 1 (T1), a type 2 (T2)

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and two type 3 (T3). These three types of copper atoms are positioned at different locations in the active centre in two different forms: a mononuclear copper centre and a trinuclear copper cluster located 12 Å apart [24].

The first of these, type1, forms a mononuclear copper centre and is the initial acceptor of electrons formed during the substrate oxidation reactions catalysed by laccase, and the redox potential of the enzyme also depends on it. In addition, T1 gives the enzyme its blue colour. Typically in fungal laccases, this copper atom is bound to a histidine residue and one cysteine residue. Bacterial and plant laccases show a slightly different mechanism, in which the copper ion is coordinated to two histidines, one cysteine and an additional axial bond to one methionine, resulting in a tetrahedral geometry. Charge transfer from cysteine to Cu (II) results in an intense absorption band at 600 nm which corresponds to the blue colour and hence the name 'blue copper'. The redox potential (E0) of a type 1 copper molecule is an indicator according to which laccases can be divided into three categories: low, medium and high potential enzymes. Bacterial and plant laccases usually have a low redox potential, in contrast to fungal laccases, which are most often categorised as having a medium and high potential. Enzymes with medium redox potential, are usually produced by *Ascomycota* and *Basidiomycota* fungi, while those with high potential are synthesised by white rot fungi [24–28].

One of the essential functions of type T1 copper is to oxidise the substrate by receiving electrons and transferring them via the cysteine-histidine pathway to a trinuclear, cluster, conserved in all MCOs, where one oxygen molecule is reduced to two water molecules The trinuclear cluster consists of binuclear type III copper atoms and mononuclear type II copper atoms A Cu T2 atom with much lower light absorption is coordinated by two histidine molecules and one water molecule. Type III copper shows strong absorption at 330 nm as a result of charge transfer from the μ-hydrox bridge (μ-OH) to type III Cu (II). The T3 atom is coordinated by three histidine residues and one water molecule [24,29,30]. The redox potential (E0) of the type 1 copper molecule is an indicator according to which laccases can be divided into three categories: low, medium and high potential enzymes. Bacterial and plant laccases usually have a low redox potential, in contrast to fungal laccases, which are most often placed in the medium and high potential categories. Enzymes with medium redox potential, are usually produced by *Ascomycota* and *Basidiomycota* fungi, while those with high potential are synthesised by white rot fungi [26].

3. Occurrence and function of laccases

Laccases are commonly synthesised by living organisms, including bacteria, plants, insects and fungi, and are involved in various biological processes, different depending on the organism producing them [22,27,31].

Producers of prokaryotic laccase can include gram-positive and gram-negative bacteria living in different environments, among which are representatives of Streptomyces: S. *lavendulae*, S.*cyaneus*, *Ralstonia solanacearum*, *Sinorhizobium meliloti*, *Bacillus subtillis*, Marinomonas mediterranea and *Escherichia coli*. The first bacterium in which laccase was discovered was *Azospirillum lipoferum*, and currently the best characterised is laccase CotA synthesised by *B. subtillis* [32–34].The cellular localisation of bacterial laccases is species-dependent and depends on the function performed. Although intracellular laccases predominate, laccases secreted outside the cell have also been recognised as in several species of *Streptomyces* bacteria such as *S. psammoticus*. Extracellular laccase has also been described in bacteria of the genus Serratia and *Bacillus* [18,35–37]. Intracellular laccases are responsible for neutralizing toxic by-products released in biochemical reactions. Under natural conditions, bacterial laccases are also involved in pigmentation, morphogenesis, toxin oxidation and protection from UV light, cross-linking of envelope proteins in bacterial spores, among others [18,38–40].

Most of the available literature data on laccase-synthesizing fungi concerns species of the genera *Basidiomycota a*nd *Ascomycota*. These enzymes are glycoproteins that usually act extracellularly; however, there are exceptions, such as the laccases of white rot fungi,

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which are also active intracellularly [21,40]. One of the main functions of fungal laccases is the breakdown of lignocellulose, which affects the carbon cycle in the biosphere. Fungal lacases also have more specialised functions, e.g. participation in the synthesis of pigment that protects fungal spores from factors such as high temperature or UV radiation or the synthesis of antibacterial compounds [41,42]. Phytopathogenic fungi, have been shown to produce laccase to inactivate plant defence mechanisms. In the case of *Cryptococcus neoformans*, laccase is considered a virulence factor. Immunocompromised individuals, such as those with AIDS or patients taking high levels of corticosteroids, are particularly vulnerable. The enzyme is thought to convert host catecholamines into melanin which promotes protection of the pathogen and allows it to cause more damage to the host [43,44].

Plant-derived enzymes are extracellular glycoproteins secreted into the apoplast due to the carbohydrate fragment present in their structure. They typically have a molecular weight of 60 to 150 kDa and exhibit a low redox potential [21,40]. All plant laccases have one common function, which is to participate in a multi-enzymatic system involved in the synthesis of lignin in wood cells, responsible for maintaining the structure and rigidity of the cell wall It is found in xylem, where it is involved in the initial phase of lignin biosynthesis by oxidising monolignols [45,46]. This enzyme is also involved in the repair mechanism of damaged plant tissues. Above this, its participation can also be seen in plant defence processes, iron accumulation and the polymerisation of phenolic compounds [21,47]. In the plant family Anacardiaceae found in resin ducts, indicating a defensive function of the enzyme against herbivores, predators and microorganisms. In the common radish (*Arabidopthis thaliana*), on the other hand, the aforementioned enzyme is involved in the polymerisation of flavonoids needed for seed coating and in the production of brown polymers with a putative protective function. In green algae present in both aquatic and terrestrial environments, the main role is the detoxification of phenolic compounds, participation in the synthesis of cell walls and UV-absorbing compounds, as well as in nutrient acquisition [21,48].

Insects are the best known laccase producers in the animal world. The presence of this enzyme has been found in insects of the order *Hymenoptera*, *Diptera*, *Lepidoptera* and *Coleoptera* [2,49–51]. Laccase is localized intracellularly in most insects and has a molecular weight of 70 to 100 kDa. Compared to plant and fungal enzymes, in insects laccase has an extended N-terminal region The main role of laccase produced by insects is its involvement in epidermal synthesis, wound healing, morphogenesis and immune system development [2,21,52,53]. In insects, laccase, located in the intestinal cells, plays a defensive role against toxic lignin derivatives entering the host organism as a result of its consumption of plant products. In these organisms, the aforementioned enzyme is also involved in epidermal hardening in larval forms, pupae and in adults, as in *Drosophilia virilis* [3]. In insects, there are two main forms of laccase: laccase-1, which is present in the salivary glands, midgut, *Malpighian tubules* and epidermis, such as in *Manduca sexta*, playing a protective role by oxidising toxic compounds ingested by the insect, and laccase-2 involved in the hardening of its epidermis. Furthermore, in the gut laccase is involved in the production of melanin, an immune system response to the presence of parasites [21,54,55].

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Table 1. Sources and biochemical characterization of laccases.

Type of source organisms	Source/ organism	MW, subunit structure	Optimum pH and temperature	Substrate (the most efficient)	Regulation of activity/synthesis	References
Plant	*Tetracystis aeria* SAG 89.80	220 kDa, hetero-oligomer with the composition AB2: two polypeptides, A (*110 kDa) and B (71 kDa) both being highly N-glycosylated	pH 2,5	ABTS		[56]
	Leucaena leucocephala	~220 kDa, heterodimer containing two subunits of 100 and 120 kDa.	pH 7.0, 80 ^{0}C	Catechol	Mn2+, Cd2+, Fe2+, Cu2+ , Ca2+ and Na+ activated laccase ; Co2+, Hg2+, DTT, SDS and EDTA showed an inhibition of laccase activity.	[57]
	Pistacia atlantica Desf.	60 kDa	pH 7.5, 45 °C	DMP	Cu2+ showed the stimulatory effect on the activity, Zn2+ and Mg2+ - have a stabilising effect of Hg2+ Fe2+have an inhibitory effect	[58]
Bacteria	*Bacillus amyloliquefaciens* B10	30.9 kDa – (predicted)	pH 6.0–8.0, 40 °C,	aflatoxin B1 (AFB1)	Cu2+, Co2+, Fe3+, Mn2+, and Zn2+ significantly reduced the degradation activity of AFB1 while Na+ and K+ slightly reduced the degradation rate of AFB1	[59]
	Enterococcus faecium A2	50.11 kDa.	pH 6.0 and 80°C	ABTS	Cr2+, Cu2+, Fe and Ag+ showed the stimulatory effect on the activity	[60]
	Bacillus sp.	MW: 63–75 kDa	pH 7/40 °C	ABTS/		[61]
Fungi	*Trichoderma harzianum* S7113,	two isoenzyme: LacA, LacBs 63 kDa (LacA) and 48 kDa (LacB)	pH 3.0, 50°C (LacA) pH 2.5, 50°C (LacB).	ABTS	Mg2+, Zn2+, K+, and Ni2+ activated laccase Hg2+ and Pb2+, β-mercaptoethanol, EDTA, and SDS, sodium azide showed an inhibition of laccase activity	[62]
	Ganoderma leucocontextum	65.0 kDa/monomer	pH 3.0, 70 °C	guaiacol	Ca, Cu i Zn showed the stimulatory effect on the activity	[63]
	Agrocybe pediades	55–60 kDa/monomer	pH 5.0 45 °C	2,6-DMP		[64]
Insect	*Plutella xylostella*	66.09 kDa	pH 3.0 and at 35°C.	ABTS	Cu2+ increased laccase activity	[65]
Crabs	*Chiromantes haematocheir*	67.708 kDa (deduced)/monomer	2,6-DMP.	presence of CuSO4 in culture medium was essential for laccase activity		[66]

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5. Applications of laccases

According to Business Communication Company (BCC) Research, the market of enzymes for industrial applications is expected to grow from USD 5.5 billion in 2018 to USD 7.0 billion in 2023 [67].

Laccase was first used industrially in the 1990s , and several laccases are now commercially available. Commercially available laccases come from *T. versicolor*, *A. bisporus*, *P. ostreatus* and *Rhus vernicifera* [40,68]. Almost all commercially available laccases are derived from fungi because such enzymes are secreted extracellularly in response to simple inducers, making their production and purification are relatively simple [6]. Although these organisms have a fairly slow growth rate and the enzymes they produce are unstable under conditions of high temperatures, alkaline stress and high salt content, are used in most industrial processes. The reason for this is that fungal laccases have a much higher redox potential than bacterial laccases. New laccases of bacterial origin are currently being sought, as they show better stability in a wide range of pH, less susceptibility to inhibitory factors such as salinity or the presence of metal ions, and are also resistant to hot temperatures. In addition, bacterial laccases, compared to fungal laccases, are quite easily subjected to all genetic engineering and protein engineering treatments [69].

White rot fungi (WRF) are a significant source of laccases. Laccase, along with lignin peroxidase and manganese peroxidase, is the main enzyme associated with the ability of WRF to degrade lignin [70,71]. Lignocellulose is the most common source of carbon on earth and accounts for more than 60% of total plant biomass [67]. This includes, but is not limited to, sawdust and paper mill waste, waste paper, agricultural residues (straw, peelings, cobs, stems, nutshells, non-food seeds and bagasse), household waste (including sewage), residues from the food industry and solid municipal waste. Lignocellulosic feedstocks are renewable resources that can potentially be used to synthesize fine chemicals such as biofuels, cellulose and paper, enzymes, composites, animal feed [72]. Unfortunately, lignocellulose is a polymer material with high resistance to degradation, thanks to lignin, a polymer composed of aromatic alcohol monomers [73]. Enzymatic delignification with laccases depolymerizes lignin, making the resulting biomass more susceptible to subsequent attack by cellulolytic enzymes. The advantages of the enzymatic pre-treatment of lignin compared to other methods include the production of less by-products, including those toxic to the environment, low energy input, significant biomass conversion and mild operating conditions [72,74,75]. The laccases of the halophilic bacteria *Aquisalibacillus elongatus* and *Chromohalobacter salexigens* showed the ability to delignify beet pulp and almond shell biowaste, respectively. The enzymes were highly stable extracellular laccases, resistant to the presence of organic solvents, salts, metals, inhibitors and surfactants in the reaction environment, and showed high catalytic efficiency in a wide range of phenolic and non-phenolic substrates differentiated in terms of structure and redox potential. Laccases can be an alternative to chemical methods of lignocellulosic fiber processing, extraction of lignocellulosic biowaste or delignification of lignin and lignin-derived industrial waste [76,77].

Processing of plant biomass, delignification of agro-industrial materials leads to the simultaneous production of glucose, which can be further used to produce bioethanol [72,75]. During the production of bioethanol from lignocellulose, in addition to a mixture of sugars, phenolic compounds are also formed that inhibit yeast fermentation, and thus the production of alcohol. To increase fuel ethanol production from renewable feedstocks, *Trametes versicolor* laccase was expressed under the control of the PGK1 promoter in *S. cerevisiae* to eliminate phenolic compounds from lignocellulosic hydrolysates [32,78,79].

Laccase is an important biocatalyst with a huge potential for industrial and biotechnological applications in the food and textile industries, organic synthesis, biosensors, biodegradation and bioremediation. Laccase participates in the direct or indirect oxidation of organic compounds with the simultaneous reduction of molecular oxygen to water, i.e. the purest reaction co-substrate [80]. The main property of laccase, used in various industries, is its ability to generate free radicals from a suitable substrate. The resulting

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secondary reactions are responsible for the versatility of the laccases in producing so many diverse products [78].

In the paper industry, laccase is mainly used for pulp delignification and paper deinking, as well as improving the properties of pulp fibers and the tensile strength of paper sheets [81]. The use of laccases in the bleaching of kraft pulp may result, in addition to a significant reduction in environmental contamination with chemicals, also in higher yields of pulp and energy savings [32].

Laccases play an important role in textile processing such as bio-bleaching of fibers (cotton and wool), denim washing, wool washing, dye synthesis and wastewater treatment. [82–88]. Laccase is an effective alternative to the most common whitening agent, hydrogen peroxide [10,81,89].

The first commercial laccase-based product used in the textile industry was DeniLite, which was launched by Novozymes in 1996. Since then, a number of laccases have been investigated for their applications in the textile industry [88].

In the food industry, laccase has been used, among others, in for stabilizing wine, clarifying fruit juices, baking or producing pectin from sugar beets [31,32,44,81,90–93]. Laccase used in the clarification of fruit juices oxidizes most of the phenols in the juice, minimizing turbidity and increasing its stability [94–97]. Since the use of laccase as a food additive is still not allowed, this enzyme is used in the food industry in an immobilized form [32,98,99].

Laccase participates in the formation of new bonds between easily oxidized natural substrates, which leads to the formation of new hybrid molecules [80,100–103]. The ability of laccases to polymerize allows for the ecological synthesis of organic compounds, e.g. including antibiotics, amino acids, antioxidants and cytostatics [67]. Laccase-generated radicals can also cross-couple with another molecule where reactive intermediates are trapped, resulting in new compounds [104].

In recent years, laccase has aroused great interest as a potential anticancer and antiviral therapeutic agent. The antiviral effect of laccases isolated from *Agaricus placomyces*, *Pleurotus eryngii* and *Pleurotus* o*streatus* has been described against HIV-1 and HCV [105–107]. The antiproliferative effect of the enzyme isolated from *Abortiporus biennis*, *T. versicolor*, *Trametes mongolicum* and A. *cylindracea* against HepG2 hepatoma cells and MCF7 breast cancer cells was observed [108–112].

Recently, the use of laccases as biosensors for the determination of xenobiotics present in samples of various origins, from the food industry to wastewater, has been reported [113–118].

A very wide range of bioremediation processes use laccase to protect the environment from damage caused by industrial wastewater. The research conducted in recent years has been intensive, and most of it resulted from the large variety of laccases, their usefulness and very interesting enzymology emerging impurities [78] such as nonylphenol, triclosan, bisphenol A, ethinylestradiol, diclofenac, 2,4-dichlorophenol and others [119–147]. In the next chapter, we will summarize the current knowledge on the processes of removing pollutants of various origins from waters with the use of laccase.

Laccases show promising potential in a variety of environmental and industrial applications, which will be listed in the Table 2.

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Table 2. Laccases application.

Application	Source organism	Activity	References
Pulp and paper industry	*T. versicolor*	demethylated and delignified the sulphate pulp	[148]
	Bacillus sp.	deinking of old newsprint and the biological bleaching of eucalyptus kraft pulp	[149]
	Fusarium equiseti VKF2	biological bleaching of newspaper waste	[150]
	T. versicolor	immobilized laccase on copper ferrite magnetic nanoparticles (CuMNPs) improve the delignification	[151]
Textile industry	*Myceliophthora thermophila*	altered the shape of wool yarn by laccase-assisted tyrosine grafting	[152]
	C. unicolor	biocatalyst to transform 8-anilino-1-naphthalenesulfonic acid into a water-soluble green dye with antibacterial and anti-allergic properties and high dyeing efficiency of wool fibers.	[80]
	Brevibacillus agri	bleaching denim and decolorizing water-soluble azo dyes	[153]
	Achromobacter xylosoxidans HWN16 *Citrobacter freundii* LLJ16	biocleaned and discolored jeans discolored the wastewater after washing the fabrics.	[154]
Food industry	*Bacillus atrophaeus*	recombinant laccase covalently immobilized on magnetic iron nanoparticles, and then used them to remove phenols and clarify plant juice samples	[90]
	T. versicolor	covalent conjugation of bovine serum albumin and sugar beet pectin (SBP) improve the stability of emulsifying beet pectin	[155]
	recombinant laccase from the fungus *Pleurotus pulmonarius* expressed in *Pichia pastoris* X33	degrade mycotoxins zearalenone and aflatoxin B1	[156]
Synthetic chemistry	laccase *Pleurotus ostreatus* immobilized on the CuFe2O4 nanocomposite	synthesize arylsulfonylbenzenediols through oxygen oxidative coupling between benzenediols and sodium benzenesulfinates	[157]
	P. ostreatus	synthesize new organic orange textile dye (N15) by transforming 2-amino-3-methoxybenzoic acid	[100]
Cosmetic industry	*T. versicolor*	bleaching agents in whitening cream that degraded up to 87% eumelanin	[158]
	C. unicolor	green compound, a result of the 8-anilino-1-naphthalenesulfonic acid (ANS) oxidation reaction, with antibacterial activity against the growth of bacterial strains Staphylococcus aureus ATCC®25923TM and Staphylococcus epidermidis ATCC®14990TM commonly found on the skin, antioxidant properties and low cytotoxicity (cosmetic additive)	[80]
	Brevibacillus agri	hair dyeing	[159]
Biomedicine	*T. versicolor*	producing laccase cross-linked hydrogels based on silk fibroin and hyaluronic acid modified with tyramine	[69]
	C. unicolor	antiviral effect against human herpes virus type 1 (HHV-1) and encephalomyocarditis virus (EMCV) anticancer effect against cell lines derived from primary cervical cancer (SiHa) and its metastases to the small intestine (CaSki)	[160]
	Bacillus sp. MSK-01	anti-proliferative activity against a lung cancer cell line A549	[161]
Biosensor	*Bacillus subtilis*	detection of glyphosate; CotA laccase effectively catalyze the luminol-H2O2 reaction, creating a chemiluminescent (CL) signal	[162]
	T. versicolor	the chemiluminescent (CL) immunoassay for the detection of Escherichia coli O157:H7 in synthetic samples (spring water, apple juice, skimmed milk),	[163]
	laccase *T. versicolor* immobilized on an	producing smart packaging systems can provide food quality information the time and temperature index, TTI	[164]

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	electrospun zein fiber (ceZL)		
Bioremediation	Recombinant *Bacillus amyloliquefaciens* TCCC 111018 laccase expressed in *E. coli*	decolorize synthetic dyes azos (azofloxine, congo red and adisole black B), anthraquinones (reactive blue 19, reactive blue 5 and remazolu brilliant blueR) and triphenylmethane (crystal violet) , indigo carmine and malachil green)	[165]
	Trematophoma sp. UTMC5003	pyrene, anthracene and phenanthrene degrading	[166]
	Sphingobacterium ksn-11	transformation of diclofenac	[135]

6. Types and mechanism of removing xenobiotics from waters

In the aquatic environment in Europe, based on the NORMAN network, at least 700 substances classified into 20 classes have been identified. Substances that enter aquatic ecosystems as a result of human activities, even in small amounts, can bioaccumulate and potentially threaten the life and health of humans and animals [9].

The development of analytical techniques has made it possible to notice the presence of many new compounds in drinking, ground and surface waters[167]. New groups of contaminants include compounds such as: human and veterinary antibiotics (e.g., erythromycin, amoxicillin, chloramphenicol), anti-inflammatory drugs (e.g. ibuprofen, diclofenac, acetaminophen), psychiatric drugs (e.g. diazepam, carbamazepine), β-blockers, lipid regulators, X-ray contrast agents, body care products (e.g. phthalates, benzophenone), endocrine disrupting compunds (EDCs) (e.g. 4-octylphenol, estrone, 17β-estradiol, 17α-ethinylestradiol, progesterone, 4-nonylphenol, bisphenol A (BPA), hormones and steroids (e.g. estradiol, estrone, estriol), perfluoronated compounds (PFCs), surfactants and surfactant metabolites (e.g. alkylphenol ethoxylates, 4-nonylphnol, 4-octylphenol, alkylphenol carboxylates), flame retardants (e.g. polybrominated diphenyl ethers (PBDEs), tetrabromo bisphenol A, plasticizers, industrial additives and agents (chelating agents (EDTA), aromatic sulfonates), antiseptics triclosan, chlorophene, esters of p-hydroxybenzoic acid (parabens) and others. In general, pharmaceuticals and EDCs are the largest group of contaminants [9,167–169].

Laccases (EC 1.10.3.2), using the redox capacity of copper, oxidize a whole range of organic and inorganic substrates. The range of substrates amenable to catalyzed reactions can be extended by mediators [67,170–173].The range of oxidized substrates varies from laccase to laccase [6,174,175].

The use of laccase in bioremediation, apart from the fact that it works on a wide range of substrates, is also justified by the fact that it catalyzes the four-electron reduction of molecular oxygen to water with one-electron oxidation of the reducing substrate, without producing hydrogen peroxide, thus creating a less destructive environment than other similar oxidases such such as lignin- and manganese-dependent peroxidases, which require hydrogen peroxide to destabilize enzymes [6,32]. The redox potential of laccase plays a major role in their reactivity. Wide differences between the redox potential values of different laccases were observed. Redox potential values lie between 0.43 V and 0.79 V. The low potential group includes laccases from trees, e.g., *Rhus vernicifera* with a potential of the T1 site of about 430 mV. The high potential laccases (e.g., those from *Trametes* (*polyporus*, *coriolus*) *hirsuta* (*hirsutus*), *T. versicolor*, *T. villosa*) all have a potential of the T1 site of about 780 mV [176,177]. Therefore, the search for new sources of laccases and their biochemical characterization is necessary in the further development of biological methods of wastewater treatment of various origins.

6.1.1. Dyes

There are about 10,000 different textile dyes, with a global production of more than 700,000 tonnes per year [8]. Dyes are present in many areas of human life. They have many applications, from the textile, tanning, cosmetics and food industries to medicine and veterinary medicine. The source of synthetic organic dyes in the aquatic environment are

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industry (including 20% of pollution comes from the textile industry and households (food dyes, e.g. tartrazine or brilliant blue, improperly disposed of unused medicines, hair dyes, cosmetics, household chemicals: shampoos, soaps, washing powder [8,178,179]. The textile industry uses large amounts of drinking water to produce fibers and thus releases huge amounts of wastewater [179].

In 1865, while trying to produce quinine, William Henry Perkin unexpectedly synthesized purple, the first commercialized synthetic organic dye. This accidental discovery led to a revolution in the use of dyes in industry and initiated the production of synthetic organic dyes on a global scale [10]. It is estimated that there are 100,000 synthetic organic dyes available on the market worldwide [8].

Dyes can be divided into natural and synthetic dyes. Synthetic dyes are easy and cheap to produce, come in a variety of colors and are durable, which makes them more widely used than natural dyes [179]. Synthetic organic dyes are divided into groups depending on their chemical structure (acridine, anthraquinone, azo, azine, diphenylmethane, indigoid, methine, nitro, nitroso, oxazine, phthalocyanine, thiazine, triphenylmethane and xanthene) and depending on the their use (acidic, basic, direct, dispersive, fibrous, reactive, vat and pickle)[180]. Synthetic organic dyes have been designed to be highly resistant to light, temperature, detergents and antimicrobial agents, therefore the dyes are highly soluble in water and slowly biodegrade in it [91,179,181].

Among them, the most commonly used dyes are azo dyes, which are resistant to decomposition under the influence of temperature, light or acids and alkalis, which makes their removal from wastewater very difficult [19]. Due to their genotoxic/carcinogenic potential, the annual disposal of ~4,500,000 tonnes of dyes and/or their degradation products poses an environmental and socio-economic problem [11].

A large amount of water used in industry and the loss of dyes during textile processes generate huge amounts of colored wastewater. The dyes present in wastewater absorb and reflect sunlight. Light penetration is limited, which disturbs the photosynthesis of aquatic flora, and consequently leads to a decrease in the amount of oxygen dissolved in water. Synthetic organic dyes have a toxic effect not only on flora but also on fauna of aquatic ecosystems. In fish, dyes are absorbed through the gills and skin [182]. They are then distributed throughout the body and undergo biotransformation. For example, azo dyes are degraded to carcinogenic aromatic amines by azoreductase produced by the intestinal microflora [183]. Compared to the parent form, a lot of these metabolites are more lipophilic and therefore easily accumulate in the fat of aquatic organisms and remain in the fish tissue longer. These mechanisms consequently lead to the bioaccumulation of dyes in the trophic chain [8,11].

Due to their toxicity to living organisms, these dyes should be detoxified or eliminated (usually discolored) prior to discharge into the environment [19,184]. Due to the complex structure of the dyes used and the complex composition of wastewater from various industries[9], physical, chemical and biological methods or a combination of several methods are used to remove dyes [184]. Textile wastewater contains dyes mixed with various impurities in different ranges like metals, solvents, salts, surfactants and other chemicals [8,19].

Biological decolorization of these wastewaters is an ecological and cost-effective approach that does not generate secondary wastewater [11,19,184]. Laccases have found particular application in the decolorization of dyes because they are able to oxidize both phenolic and non-phenolic compounds [184].

Myrothecium verrucaria NFCCI 4363, a brown rot fungus isolated from contaminated soil and sugar industry waste disposal sites, produces extracellular laccase capable of decolorizing wastewater. The enzyme showed maximum activity at pH 6 and 50°C. Metal ions Cd2+, Cu2+, Fe2+ and Mn2+ significantly increased laccase activity at low concentrations. Low concentrations of solvents (dichloromethane, acetonitrile and methanol) had no significant effect on enzymatic activity. The decolorization potential of *M. verrucaria* NFCCI 4363 crude laccase was evaluated using azo, triarylmethane and nitro dyes. The enzyme without mediators discolored triarylmethane dyes (bromothymol blue,

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bromocresol green and bromocresol purple) with an efficiency of about 80%. In the presence of ABTS as a mediator, laccase discolored acid fuchsin in about 70% and crystal violet in about 58%. Jawale et al. (2021) suggest that the degree of decolorization of dyes by laccase depends on the origin of the enzyme, the presence of mediators and metal ions in the reaction mixture[185].

A single laccase with a molecular weight of 41 kDa was produced by the white rot fungus *Oudemansiella canarii* EF72, an edible mushroom from Agaricales (mushrooms) commonly found in the Atlantic rainforest, Amazon rainforest and Pantanal [186]. The enzyme was stable between pH 3.0 and 8.0 for at least 6 hours. Laccase decolourized to 80% Congo Red in 24 hours at 30°C and pH 5.5. The azo dye Congo Red is present in significant amounts in textile and paper industry effluents and has been reported to be carcinogenic to humans and toxic to humans (Chung, 2016). The analysis of Congo red degradation products revealed that the laccase acts on the chromophore group of the dye and asymmetrically cleaves azo covalent bonds, causing effective fragmentation of the molecule and the formation of products with nitrification of the NH_2 group and loss of the SO_3 group. The released naphthalene derivatives containing nitrified and/or hydroxyl groups were attributed by the authors of the study to the oxidation of amino groups present in Congo red. In the last stage of degradation, the benzene ring opened and a fully oxygenated compound with the formula C8H3N2O8 was formed. The native laccase treatment reduced toxicity by 92.5% after 24 hours. In conclusion, *O. canarii* laccase can be used in the bioremediation and detoxification of azo dyes.

The laccase of spores of the halotolerant bacterium *Bacillus safensis* S31, isolated from the soil of a chromite mine in Iran, showed the ability to decolorize various classes of dyes. The maximum activity of the enzyme was achieved at 30°C and pH 5.0. The enzyme tolerance the presence of 10% solutions (v/v) of solvents such as methanol, ethanol and acetone and most of tested metal ions. Unlike many other laccases, NaN3 at concentrations of 0.1 and 1 mM showed a slight inhibitory effect on the enzyme activity (7.5%), which is important due to the fact that NaN3 is a toxic substance found in textile industry wastewater and dyeing. The enzyme showed the highest decolorization efficiency in relation to malachite green (87%) in the presence of ABTS and in relation to reactive black 5 (75%) in the absence of ABTS. In conclusion, the research by Siroosi et al. (2018) proved that B. safensis S31 spore laccase can be used for bioremediation of textile wastewater containing a number of dyes due to its high tolerance to metals, salts, solvents and sodium azide [19].

6.1.2. PAH (polycyclic aromatic hydrocarbons)

The increasing rate of industrialization is gradually leading to pollution and degradation of the environment, and PAHs are among the main pollutants. Polycyclic aromatic hydrocarbons (PAHs) are aromatic hydrocarbons with two or more fused benzene rings in different structural configurations that do not contain heteroatoms or substituents. Their benzene ring systems have a wide range of physical, chemical and toxicological properties [187]. Chemically, we can distinguish low molecular weight (LMW) and high molecular weight (HMW) PAHs. Both low molecular weight (LMW) and high molecular weight (HMW) PAHs can be microbially degraded by [188]. LMW PAH are more susceptible to degradation due to the rather higher volatility and solubility of the compound [12]. Due to the stability, hydrophobicity and lipophilicity of PAH molecules, they are harmful persistent organic pollutants that are very difficult to biodegrade [12,187].

PAH includes compounds such as: anthracene, pyrene, naphthalene, acenaphthylene, acenaphthene, fluorine, phenanthrene, anthracene, fluoranthene , pyrene, benz(a)anthracene, chrysene , benzo (b) fluoranthene, benzo (k) fluoranthene, benzo(a)pyrene and others [187,189]. These compounds are widely used in various industries, e.g. for the production of dyes, pharmaceuticals, plastics, fungicides and insecticides [12].

PAHs are widely distributed in various ecosystems Lawal 2017 In the aquatic environment, PAH concentrations range widely from 0.03 ng /l (marine water; Southeast Sea

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of Japan, Japan) to 8,310,000 ng /l (domestic sewage treatment plant, Siloam, South Africa). Due to its poor water solubility and low vapor pressure, chrysene is one of the most persistent PAHs in the water column [190].

Moreover, the bioaccumulation of PAHs in fish was found to range from 11.2 ng /l (Cynoscion guatucupa , South Africa) to 4207.5 ng /l (Saurida undosquamis , Egypt). Such a PAH is benz[a]anthracene (BaA), which tends to accumulate in lipid-rich tissues [12].

Aquatic ecosystems are exposed to PAHs mainly as a result of accidental oil spills, with huge health consequences for the fish, birds and mammals living in these ecosystems. Effects of PAH exposure in fish include immunotoxicity , gill deformity, increased mortality, reproductive disruption, liver tumors, uncontrolled cell growth, reduced growth, embryo malformations, osmoregulatory imbalance, and endocrine disruption [188]. In birds, deformities and death of embryos, low fecundity, slowed growth and metabolic disturbances have been reported [191].

More and more studies report that PAHs are organic compounds with a high degree of toxicity, carcinogenicity and teratogenicity [187]. Of the more than one hundred known PAHs, sixteen have been classified as priority environmental pollutants by the United States Environmental Protection Agency (US EPA). This group includes acenaphthene, benzo[ghi]perylene, chrysene, acenaphthylene, benz[a]anthracene, benzo[b]fluoranthene, anthracene, benzo[k]fluoranthene, benzo[a]pyrene, fluoranthene, indene [1,2,3 -cd] pyrene, naphthalene, phenanthrene, dibenz[a,h]anthracene, fluorene and pyrene [188]. Among the various PAHs, benzo[a]pyrene (BaP) has the greatest carcinogenic potential .

PAHs from the aquatic environment can be removed by physical, biological and chemical processes [187]. Physical and chemical methods for PAH removal are often inefficient, labor intensive and expensive [192], in addition, through incomplete transformation of PAHs, they can lead to the formation of other stable intermediates that are more toxic than the parent substrate [12,188].

For the reasons mentioned above, interest in the development of biotechnology for detoxification of PAH pollutants has increased [166]. Bioremediation is an effective strategy for the treatment of organic pollutants, including PAHs, based on the use of microorganisms to convert pollutants into substances inert to the environment. Bioremediation, due to the relatively low cost and greater efficiency for compounds with a high degree of structural complexity, is one of the widely used technologies in the removal of pollutants [187].

The most promising class of enzymes for PAH detoxification are oxidoreductases [193–197]. Oxidoreductases include laccases , which catalyze reactions with a wide range of aromatic impurities such as phenols and PAHs as substrates [188].

The fungus *Trematophoma* sp. UTMC5003 isolated from soil in Iran could in the future be used in the bioremediation of oil-contaminated soils and waters. The isolate obtained by Moghimi et al . (2017) is capable of producing biosurfactant and laccase . The fungus degraded 70% of crude oil and 90% of aliphatic compounds and down to 56, 87 and 90% of, respectively, within 15 days. *Trematophoma* sp. UTMC 5003 produced 4U/L of laccases in the presence of petroleum as a carbon source [166].

Laccases produced by *Leucoagaricus gongylophorus* FF-2006, isolated from *Atta sexdens rubropilosa* , act in lignocellulose degradation and detoxification processes . Therefore, the work proposed the use of *L. gongylophorus* laccase (Lac1Lg) for the degradation of anthracene, fluorene, phenanthrene, fluoranthene and pyrene without the use of mediators. Degradation reactions were performed in an aqueous buffer solution at pH 6.0 in the presence of Tween-20 and a 50 U/L laccase preparation at 30°C and with constant magnetic stirring. Within 24 hours, 72 ± 1% of anthracene, 40 ± 3% of fluorene and 25 ± 3% of phenanthrene were biodegraded. Lac1Lg degraded anthracene to form anthrone and anthraquinone, which are interesting compounds for industrial applications. Metabolites for degradation of the remaining PAHs were not observed, possibly due to their water solubility and low concentration [198].

Biodegradation of naphthalene, anthracene and 1,10-phenanthroline using *Pleurotus ostreatus* has been studied along with expression levels of laccase genes. Naphthalene was

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completely degraded within 5 days, while anthracene and 1,10-phenanthroline were biodegraded in 93.69% and 92.00%, respectively, in the incubation period of 11 and 14 days, respectively. Naphthalene was chosen as a model compound for the analysis of the metabolic pathway of PAH degradation. Based on the obtained metabolites, the researchers proposed the course of PAH degradation by *P. ostratus* and its enzymes, naphthalene dioxygenase and laccases . Naphthalene was first degraded to α- and β-naphthol, which were then metabolized to salicylaldehyde. This compound was oxidized to salicylic acid. Salicylic acid was further dehydroxylated to benzoic acid[199]. In addition, a higher level of degradation of naphthalene was observed compared to anthracene and 1,10-phenanthroline, further supporting the idea that low molecular weight PAHs are more easily degraded due to their higher solubility and greater bioavailability.

Torres-Farrada G et al . investigated the potential of extracellular crude enzyme extracts of *Ganoderma* sp., the white rot fungus (WRF), from urban ecosystems in Havana, Cuba, to degrade PAHs. *Ganoderma* strains degraded the three PAHs with varying efficiencies (naphthalene, 34–73%; phenanthrene, 9.4–67%; fluorene, 11.4–64%). The presence of HBT significantly increased the degradation of naphthalene, phenanthrene and fluorene. Nevertheless, *Ganoderma* sp. UH-M was able to degrade PAH with the same efficiency with and without a redox mediator, which is advantageous for practical use.

As a result of enzymatic degradation of naphthalene, 4 metabolites were detected: benzoic acid-TMS derivatives , catechol -TMS derivatives, phthalic acid -TMS derivatives and protocatechuic acid-TMS derivatives. After the degradation of phenanthrene, phthalic acid -TMS derivatives and protocatechuic acid-TMS derivatives were detected . For fluorene, 9-hydroxyfluorene, 9-fluorenone and phthalic acid-TMS derivatives and protocatechuic acid-TMS derivatives have been identified. These compounds, phthalic acid and catechol , can be mineralized by fungal enzymes. *Ganoderma* sp. UH-M is a promising candidate for bioremediation of PAH-polluted ecosystems[200].

Lasiodiplodia theobromae fungus, isolated from a PAH-contaminated soil sample at the Beijing Coking Plant in Beijing, China, was able to degrade benzo[a]pyrene (BaP). *L. theobromae* belongs to the group of botryosphaeriaceous fungi , is the causative agent of storage rot in many fruits and tubers, and is a serious pathogen of many agricultural and horticultural crops [201]. It turned out that this fungus has the potential to use BaP as a sole carbon source. Within 10 days, up to 53% of BaP was degraded. During the biodegradation of BaP, the activity of lignin peroxidase and laccase was detected, and it should be noted that the activity of laccase was 4 times higher than that of lignin peroxidase. The addition of Tween 80 (TW-80), glucose and salicylic acid slightly increased the biodegradation of BaP .

It has been reported in the literature that white rot fungi, which produce ligninolytic enzymes, can oxidize PAHs by generating hydroxyl radicals by donating one electron, which oxidizes the PAH ring, which leads to the formation of quinones and acids, which are finally decomposed to CO2 and H2O by hydrogenation, dehydration and so on. *L. theobromae* LAC can catalyze the one-electron oxidation of PAHs such as anthracene (ANT) and BaP. The effectiveness of LAC increases in the presence of mediators such as 1-hydroxybenzotriabol (HBT) or 2,2'-azinobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) [201].

Pozdnyakova et al. (2018) note that laccase can catalyze the initial attack on the PAH molecule, which leads to the formation of quinones, and their further oxidation is provided by the peroxidase, which ultimately leads to PAH mineralization. A. bisporus , which produced only laccase , metabolized phenanthrene and anthracene to give the corresponding quinones as the dominant metabolites. No downstream products of these compounds were detected. Thus, the affiliation of fungi to different ecophysiological groups and the conditions of their cultivation affect the composition and dynamics of the production of the complex of ligninolytic enzymes and the completeness of PAH utilization [202].

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6.1.3. Pharmaceutical

Worldwide annual consumption of PhACs is estimated at 100,000 tonnes or more, with the trend increasing due to disease and aging [15].

PhACs are natural or synthetic compounds used in medicine and veterinary medicine to diagnose, prevent and treat many diseases. A wide range of medicines for humans and animals, such as non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, synthetic hormones and others have become essential to the health and well-being of the population, and what thus extending their life expectancy. PhAC consumption is expected to increase in the coming years due to the aging of the population and the improvement of health standards [13,14]. Antibiotics, anti-inflammatory drugs, analgesics, antidepressants, antiepileptic drugs, lipid-lowering drugs, β-blockers, antiulcer drugs and antihistamines are the most commonly consumed drugs [14].

Mixtures of pharmaceuticals with their active metabolites are continuously discharged into the environment through wastewater treatment plants, municipal wastewater, wastewater from hospitals, the livestock and pharmaceutical industries, improper disposal of unused or expired pharmaceuticals, the use of manure and sludge as organic fertilizers, and leachate from landfills solid waste [14].

The persistence of pharmaceuticals in the environment has become a problem with their ever-increasing production. Potential toxicological effects on non-target organisms have been observed as a result of the continued presence of these chemicals in the environment at low concentrations (ranging from μg L-1 to ng L-1). Some of these compounds have already been placed on the second and third watch lists, as is the case with (i) the antibiotics amoxicillin, ciprofloxacin, erythromycin, clarithromycin, azithromycin, sulfamethoxazole and trimethoprim; (ii) the hormones 17-alpha-ethinylestradiol (EE2), 17-beta-estradiol (E2) and estrone (E1); (iii) the synthetic hormone norethisterone; (iv) antidepressant venlafaxine and (v) three antifungal drugs: clotrimazole, fluconazole and miconazole [13,203–205].

Overuse of PhACs and ineffective wastewater treatment systems have led to the accumulation of these residues in aquatic systems, which has been reflected in behavioral and physiological changes in the animals [206]. Pharmaceuticals that may be present in the water affect not only zebrafish organ development and function, but also its DNA, hormone secretion and other biomarkers of oxidative stress that can be used to assess the impact of pharmaceutical contaminants on marine organisms [207].

Hormones present in water bodies, even in low concentrations, depending on the type, can cause changes in sex characteristics and sex ratios, feminization or masculinization of fish, or impair the development of their immune system [9]. Silva et al. (2019) further revealed that progesterone and estradiol have an effect on zebrafish locomotor activity, with a decrease in both slow and fast swimming [208]. The animals also showed an increase in aggression, an increase that worsened their propensity to reproduce .

Ibuprofen and diclofenac are the most widely used non-steroidal anti-inflammatory drugs (NSAIDs) in the world ,that impair the cardiovascular development of zebrafish (*Danio rerio*) [209,210]. Diclofenac is a compound of low to moderate toxicity, as indicated by increased activity of enzymes oxidative stress (glutathione s-transferase (GST), catalase (CAT)) and lipid peroxidation. Diclofenac is also an endocrine disruptor (EDC).

The source of PhAC in the aquatic environment may be insufficiently well-treated sewage from the discharge from the sewage treatment plant or runoff from agricultural areas fertilized with manure from dairy cattle breeding [9]. The current concentration of pharmaceuticals released into the environment exceeds its natural ability to decompose them [14].

Several technologies have been developed over the years to remove PhAC from the environment. Due to the resistance of some pharmaceutically active compounds (PhACs), conventional wastewater treatment is unable to effectively remove them [15]. The solution to these inconveniences may be the use of bioremediation, which uses the ability of microorganisms to decompose various organic pollutants [14]. PhAC biocatalysis requires

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less energy input, operates under moderate conditions and produces fewer or no toxic by-products compared to other conventional technologies. In addition, the substrate specificity of the enzymes allows to minimize adverse side reactions, if necessary, by modifying the enzymes by genetic engineering and protein engineering[15,211] Enzymes are a promising technique for the selective removal of contaminants from water and wastewater [40].

Research conducted in recent years has evaluated the effectiveness of PhAC removal by various forms of enzymes, such as whole cell cultures, crude extracts, free and immobilized enzymes, enzymes obtained by heterologous expression or site-directed mutagenesis [15].

Purified extracellular laccase with a mass of 90 kDa secreted by *Sphingobacterium* ksn-11, isolated from the soil of agricultural fields, after immobilization was used by Neelkant et al. (2020) for the transformation of diclofenac (2-(2-(2,6-dichlorophenylamino)phenyl acetic) [135]. The purified enzyme was immobilized in sodium alginate-silicon dioxide-polyvinyl alcohol beads and evaluated for diclofenac transformation efficiency. After 4h incubation at 40°C and pH 4.5, 75% of diclofenac was transformed without mediator and in the presence of ABTS 81% of diclofenac was transformed within 90 minutes. LC-MS analysis confirmed that the immobilized laccase converted the diclofenac to 4,5-dihydroxy diclofenac and 3-hydroxy diclofenac. The presence of the mediator had no effect on the quantity and quality of the products. The authors speculate that the removal of diclofenac occurs in the following steps: i) hydroxylation, ii) ring opening reaction, iii) mineralization. The work clearly indicates the possibility of using *Sphingobacterium* ksn-11 laccase immobilized on a polyvinyl-alginate-silicon dioxide matrix for further research on the degradation of endocrine disruptors and other xenobiotic compounds.

It was recently discovered that the enzyme laccase from the white rot fungus *Trametes hirsuta* is capable of degrading chloramphenicol (2,2-dichloro-N-[(1R,2R)-1,3-dihydroxy-1-(4-nitrophenyl)propane-2- yl]acetamide) at a concentration of 0.5 mg/l for 7 days without mediators. Chloramphenicol is a broad-spectrum antibiotic pharmaceutical used to treat bacterial infections in humans and animals. It is one of the most durable thermotolerant xenobiotics among hospital waste. The use of the mediator-laccase system allowed for the effective removal of chloramphenicol in higher concentrations (10 mg/l) and shortened the time needed to completely remove the antibiotic from the reaction environment to 48 h. Laccase caused dehalogenation and oxidation of CAP to chloramphenicol aldehyde, which in MIC tests was non-toxic to the tested microorganisms (Staphylococcus aureus, Escherichia coli, Candida albicans). Taken together, the results suggest an effective use of laccase and its mediators in the bioremediation of antibiotics, the most persistent micropollutants in pharmaceutical waste [212].

Streptomyces mutabilis A17 laccase obtained by solid-state fermentation on agro-waste degraded sulfonamide antibiotics. The maximum enzymatic activity was reached at 50°C and pH 6.0 for 1 hour in the presence of 1 mM HBT (1-hydroxybenzotriazole) as a mediator. The degradation efficiency of sulfadiazine (SDZ) and sulfathiazole (STZ), sulfa antibiotics was 73% and 90%, respectively. The toxicity of laccase-treated sulfonamide antibiotics tested in the microbial toxicity test against *B. cereus* LC314797, *S. aureus* KT337489, *S. enterica* MK127926 and K. pneumoniae KF771031 was significantly reduced. *S. mutabilis* A17 laccase can be used in the enzymatic degradation and detoxification of sulfonamides from environmental pollutants [213].

6.1.4. Plasticizers

The consumption of plastics has increased significantly in the last few decades. As a consequence of the huge market demand, the production of plastics increased from 1.7 million tons in 1950 to 438 million tons in 2017 [214]. A fundamental issue in the current use of plastic is its disposal. Only 10% of plastic waste is recycled; a significant proportion ends up in landfills or the environment[214,215].

Materials made of plastics are widely used in everyday life, mainly as food packaging. Primary packaging is typically made of polyethylene. Polymers used in the

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production of packaging are supplemented with various additives that improve their properties. Such an additive to plastics is BPA (2,2-bis(4-hydroxyphenyl)propane)[216].

BPA is a monomer in the production of polycarbonate plastics and epoxy resins, and as an additive in the production of PVC coatings, it is added to various plastics as a plasticizer. These materials are used in food storage containers, water, baby bottles, food and beverage cans [216]. BPA is also an important ingredient in adhesives, flame retardants, paints, protective coatings, automotive lenses, compact discs, construction materials, thermal paper, and dental sealants and composites [217]. Global production of BPA exceeded 4.6 million tonnes in 2012 and will grow at a rate of 4.6% annually from 2013 to 2019 [218,219].

BPA can diffuse into food and beverages. BPA migration from packaging increases during exposure to sunlight and elevated temperatures [216]. People are exposed to the adverse effects of BPA mainly through the consumption of food and beverages stored in plastic containers, bottles and cans. In living organisms, BPA mimics the structure of estrogen and binds to estrogen receptors [220]. Although BPA has been shown to have weak estrogenic activity, it can disrupt the proper functioning of the endocrine system even at extremely low concentrations.

Excessive exposure of people to BPA can lead to cardiovascular, endocrine and immunological disorders, diabetes and obesity [216]. Research has shown that BPA exhibits genotoxicity , reproductive toxicity, endocrine disrupting activity, cytotoxicity, neurotoxicity and hepatotoxicity [219]. In women, its presence in the environment has been linked to breast cancer [221], polycystic ovary syndrome , miscarriage, endometrial disorders and premature birth [9,216,217]. In men, BPA has been associated with lower semen quality, fertility, sex hormone levels and sexual dysfunction.

BPA tends to accumulate in aquatic animals; hence its bioaccumulation along the food chain. Human consumption of marine fish containing BPA may cause its presence in the human body [216]. Therefore, restrictions on the use of BPA have been introduced. Maximum Total Daily Intake (TDI) according to the US Environmental Protection Agency (US Environmental Protection Agency , 2010) is 0.05 mg/kg body weight/day, and according to EFSA and the European Commission (EC) - 0.004 mg/kg body weight/day. The EC has also set a specific migration limit for BPA in food at 0.5 mg kg-1 (European Food Safety Authority, 2015; European Commission, 2018a; US Environmental Protection Agency 2010) [220].

The release of BPA into the aquatic environment takes place in several ways, including from production plants, from wastewater as a result of incomplete treatment, from leachate from landfills, from leaching from non-recycled plastics [216]. In the surface and bottom waters of the Gulf of Gdańsk, BPA values up to 277.9 ng L-1 were recorded [222].

Biodegradation and adsorption are the main BP removal routes in wastewater treatment plants [125,223]. WRFs convert BPA through an enzymatic oxidation reaction into a much less reactive substance. The oxidized form of BPA does not bind to ERα-dependent estrogen receptors. The product of BPA oxidation catalyzed by laccase is 2,2-bis(4-phenylquinone) propane [125,126].

Two isoforms of laccases isolated by Elsayed et al. (2023) *Trichoderma harzianum* S7113 have been used in the biodegradation of BPA[62]. The isoenzymes differed in their tolerance to the presence of Na+ ions. Na+ decreased LacB activity and stimulated LacA . Lac A showed greater catalytic potential towards BPA as a substrate than LacB . LacA degraded BPA at a concentration of 20 mg/L. at pH 5.5 over 5-hour batch tests at a laccase concentration of 0.75 U/ml with an efficiency of approx. 70%.

Highly active and stable laccase was isolated from spore cells of *Bacillus* sp. GZB, a BPA-degrading bacterium. The *Bacillus* sp. GZB strain was obtained from sludge collected from an electronic waste recycling facility in Guiyu , China. Complete degradation of BPA at a concentration of 10mg/L was achieved after 30 hours of incubation. Based on the identified intermediates, the pathway of BPA degradation by bacterial laccase was proposed . Due to the oxidation of BPA by laccase , phenoxy radicals were formed in the first step , and further oxidation or cleavage of the C-C bonds of the radicals resulted in the

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formation of various organic acids. During degradation, the toxicity of BPA, tested against *Photobacterium phoreum* , gradually decreased. Exceptional laccase resistance *Bacillus* sp. GZB to changes in temperature and pH is due to the structure of the spore cells, which can serve as a matrix in immobilization. This enables the use of laccase as a natural biocatalyst in wastewater treatment to remove the toxicity caused by BPA[224].

The accumulation of synthetic plastics is a serious problem for the environment and human health. The most commonly used homopolymers in the production of plastics is polyethylene (PE) [214,215].

The rapid increase in plastic production and waste volumes requires the development of effective plastic waste management solutions [214]. Plastic waste in the environment is naturally broken down by photo-, bio- and thermo-oxidative depolymerization as well as friction [215]. All types of abandoned plastic products are exposed to the environment, creating plastic particles of various sizes that seriously pollute water resources and pose a serious threat to aquatic and marine organisms [225,226].

Biodegradation is necessary for water-soluble or water-immiscible polymers because they end up in water streams that cannot be recycled or incinerated [226]. Unfortunately, petroleum-derived (petro)polymers are extremely resistant to natural pathways biodegradation. Their complete decomposition in the natural environment can take over 50 years [215]. Enzymatic degradation of plastics begins with the adsorption of enzymes on the polymer surface followed by hydroperoxidation /hydrolysis of the bonds. The sources of enzymes that break down plastics can be found in microorganisms from various environments and in the digestive intestines of some invertebrates [215].

PE is a high molecular weight polymer linked by a C-C single bond [227]. PE is one of the most inert plastics, and its resistant nature is due to its high molecular weight, complex three-dimensional structure and hydrophobic properties that make it difficult for microorganisms to access it [226].

Santo proved that the breakdown of polyethylene macromolecules can be catalyzed by enzymes secreted by microorganisms and indicated that laccase can affect polyethylene molecules by creating carbonyl groups and thus reducing the molecular weight of PE molecules [228].

Trichoderma harzianum laccase and manganese peroxidase from landfill soil in Shivamogga district were able to degrade PE to form carboxylic acid groups, aldehydes, aromatic compounds, alcohols, esters, ethers and alkyl halides [226].

In the work of Zhang et al . (2022) recombinant laccase from *Acinetobacter baumannii* Rd-H2 was obtained isolated from a grain and food storage pest [227]. The recombinant AbMCO enzyme led to the occurrence of basic structural changes on the surface of the PE film as a result of the appearance of a carbonyl group formed by the presence of oxygen atoms in the carbon chain of the polymer. The enzyme expressed in E. coli had a temperature optimum of 45 ° C and a pH optimum of 4.5. It showed good stability at 30–45 ° C. Zhang 2022 The bacterial treatment breaks the chain of the PE polymer, which reduces the molecular weight and increases the hydrophilicity of the PE polymer.

6.2. Mechanism of removing xenobiotics from waters

There are two types of laccase-catalysed reactions: catabolic and anabolic. The catabolic pathways involve mediators, i.e. small organic substances that facilitate the course of the redox cycle. In contrast to anabolic processes, catabolic processes are mediated by laccases derived from white or brown wood rot fung [6,229].

Jeon et al. (2013) list two factors that may affect the course of reactions catalyzed by laccase. These are: (i) the redox potential of the enzyme and (ii) the stability of the phenoxy radicals formed as a result of oxidation by laccase. Fungal laccases, responsible for catabolic processes and wood decomposition, show a high redox potential compared to laccases involved in anabolic processes [1,9]. As a result of the decomposition of lignin present in wood, phenols are formed, e.g. syringaldehyde and acetosyringone, which are characterized by high structural stability and a long half-life of the corresponding

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radicals. The listed properties of the products resulting from the decomposition of wood by laccase shift the course of the reaction towards catabolic processes [6]

Bioremediation and detoxification of xenobiotics present in the environment is an example of the use of laccase's ability to catalyze both catabolic and anabolic reactions. [6,229,230].

Synthetic dyes are high-molecular organic compounds with a complex structure. Bankole et al. (2019) for decolorization of thiazole yellow G used filamentous fungus *Aspergillus niger* LAG [231]. A significant role in the degradation of this dye was attributed to laccase. The fungus showed significant induction of laccase (71%) and lignin peroxidase (48%) in the presence of xenobiotic. Probably the scheme of dye decomposition is as follows: (i) lignin peroxidase catalyzes asymmetric cleavage of the bond in thiazole yellow G, two sodium 6-methyl-2-phenyl-1,3-benzothiazole-7-sulphate molecules are formed (ii) sodium 6-methyl-2-phenyl-1,3-benzothiazole-7-sulphate molecules are desulfonated and demethylated by laccase, resulting in 2-phenyl-4,5-dihydro-1,3-thiazole. These products showed less toxic potential.

As mentioned earlier, polymerization reactions resulting from oxidative coupling are also involved in the removal of organic pollutants. These processes lead to the formation of adducts or copolymer products that are insoluble in the aqueous environment. To remove pollutants that polymerize, the wastewater is then filtered or sedimented[230,232].

Triclosan is an antimicrobial agent used in pharmaceutical and personal care products. Due to its hydrophobicity, structural stability, and antibacterial properties, triclosan is a compound that is difficult to remove from water and resistant to biodegradation [233–235].

Sun et al. (2019) proved that the way to remove triclosan from the environment is the enzymatic activity of *T. versicolor* laccase supported by Cu2+ ions[236]. The enzymatic reaction mainly produces triclosan dimers, trimers and tetramers. A possible transformation mechanism of the xenobiotic has been proposed: (i) laccase catalyzes the oxidation of triclosan to generate intermediate phenoxy radicals, (ii) phenoxy radicals conjugate to each other via a radical covalent bond of CC and COC to form oligomers and polymers. The presence of Cu2+ enhanced the self-polymerization of triclosan by forming more triclosan oligomers relative to Cu2+-free oligomers, which is probably attributed to increased laccase activity and stability with Cu2+ present in the reaction medium.

Acetaminophen is one of the difficult-to-remove types of pharmaceutical contaminants that have been noticed in municipal water. Wang et al. (2018) presented a potential method of removing acetaminophen from water based on the laccase-catalyzed oxidative coupling reaction with the formation of insoluble products that are easily separated from the liquid by filtration [237]. In order to increase the efficiency of xenobiotic removal, a dual pH optimization strategy was used (enzyme solution at pH 7.4 is added to substrate solution at pH 4.2). The increase in enzyme activity was attributed to favorable electron transfer. The neutral state favored electron transfer between the substrate and the Cu T1 site of the active center of the laccase, and the acidic state allowed electron transfer from the T1 site of Cu to the TNC. Within 1000 s, approximately 93% of acetaminophen was converted into biologically inactive polymerized products, and some of them precipitated from water. The course of the reaction consisted of laccase-catalysed oxidation, radical polymerization and precipitation. The laccase-catalysed oxidation is the rate-limiting step of the entire process. The higher rate of oxidation of substrates by laccase as part of a dual pH optimization strategy led to much faster formation of acetomiphene dimers, trimers and tetramers that could naturally precipitate from water.

Das et al. (2018) investigated the pathway of BPA degradation by the laccase of *Bacillus* sp. GZB spores [224]. They noted the presence of BPA degradation intermediates such as 4-hydroxybenzaldehyde, benzoic acid, 2-hydroxypropanoic acid, 2-methylbutanoic acid and 3-methylbutanoic acid. In other studies where other microorganisms were the source of laccases, the formation of other intermediates was identified. In the

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reaction of BPA with *Trametes villosa* laccase, phenol and 4-isopropenylphenol are formed [238], with immobilized *Coriolopsis polyzona* laccase on silica nanoparticles: phenol, 4-isopropenylphenol and 4-hydroxyisopropylphenol [239], with laccase *T. versicolor*: ethylbenzene, p-xylene, cyclohexanone, and 1-methyl-4-isopropenyl-2-cyclohexenylene [240], with *Phanerochaete chrysosporium* laccase: 2,2-methylenediphenol, bis(4-hydroxyphenyl)methane and p-(benzyloxy)phenol [128], with *T. versicolor* laccase immobilized on *Hippospongia communis* spongin scaffolds : phenol, catechol, dimers [241]. In the case of BPA degradation by *Pseudomonas putida* YC-AE1, the intermediates are 4,4-dihydroxy-alpha-methylstilbene, p-hydroxybenzaldeyde, p-hydroxyacetophenone, 4-hydroxyphenylacetate, 4-hydroxyphenacyl alcohol, 2,2-bis(4-hydroxyphenyl)- 1-propanol, 1,2-bis(4-hydroxyphenyl)-2-propanol and 2,2-bis(4-hydroxyphenyl) propanoate. [242]. In general, the biodegradation pathway of xenobiotics, the quantity and quality of the final products depends on the source of the laccase.

The presence of mediators has a significant impact on the course of reactions catalyzed by laccase. Mediators are low molecular weight, water-soluble chemicals with a high redox potential (approx. 900 mV) that can act as one-electron shuttles between the enzyme and the compounds to be oxidized [91,181]. The most effective mediators are N-heterocyclic compounds containing N-OH groups, such as hydroxybenzotriazole (HBT), 2,2,6,6-tetramethylpiperidin-1-yloxy (TEMPO), N-hydroxyphthaimide (HPI), N-hydroxyacetanilide (NHA) and violuric acid (VLA) [67].

Degradation processes are based primarily on laccase - mediator systems (LMS), LMS induces the cleavage of oxidative bonds present in the structure of xenobiotics. These systems effectively break down several different types of xenobiotics, including synthetic dyes, NSAIDs, pesticides and polycyclic aromatic hydrocarbons (PAHs) [173,243–248].

In the studies by Daassi et al. (2016,) using the crude laccase enzyme of the fungus *Coriolopsis gallica* (BS54) [KJ412304], to BPA degradation. The final product of BPA degradation was b-hydroxybutyric acid. Oxidative cleavage of BPA led to the formation of b-hydroxybutyric acid in the absence of HBT and tartaric acid in the presence of HBT. Final products were significantly different from that after treatment with BPA with the laccase/HBT system because the mediator is also oxidized to give other degradation and by-products. Tartaric acid and pyroglutamic acid probably originated from degradation of HBT. The formation of tartaric acid and b-hydroxybutyric acid could be the result of oxidation of methyl groups on the propane moiety of the BPA molecule under the action of laccase. Organic acids such as carboxylic or polycarboxylic acids (tartaric acid and b-hydroxybutyric acid), which are commonly found as intermediates in metabolic pathways, are usually formed by the fusion of aromatic rings [7].

The action of mediators is beneficial because it allows laccases to oxidize large molecules, such as lignin, without the need for direct enzyme-polymer interaction, and to increase the range of substrates with compounds with redox potentials exceeding their own. The use of mixtures of mediators enhances their synergistic effect and increases laccase activity [158,249].

7. Methods of increasing the productivity and activity of laccases

The trend is an increase in the production of enzymes related to the intensive development of industry, bioremediation or synthetic chemistry. This leads to the search for laccases with greater activity and stability under extreme conditions of temperature, pH , etc. These studies are aimed at improving the economics of the processes compared to those currently available. The main goal is to reduce enzyme production costs and improve their properties [67]. The potential of laccases to degrade xenobiotics can be promoted by improving enzymatic catalytic characterization using protein engineering and other genetic engineering methods [72].

Basidiomycetes, Ascomycetes and *Deuteromycetes* with high redox potential are used to produce biotechnologically important laccases [91,250]. Laccases of different origin

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break down pollutants in a different way. Therefore, the result of their enzymatic activity are products that differ in structure, chemical and physical properties, and with varying degrees of toxicity. For example, as a result of the degradation of BPA by laccases *Bjerkander adusta* and *T. versicolor* , glycerol is formed, and *Coriolopsis gallica* – b- hydroxybutyric acid [7]. Therefore, it is important to search for new sources of laccases and their physiological characteristics in order to optimize the culture conditions, as well as to isolate enzymes and their biochemical characteristics in order to develop rational methods of protein modification and introduce changes favorable for the course of biodegradation processes.

7.1. Improvement of catalytic activity of laccases

In recent years, researchers have used methods such as optimization of reaction conditions, including the search for reaction mediators or inducers, the use of surfactants, immobilization of enzymes on matrices with various properties, and modifications of catalytic activity and substrate specificity using molecular biology and engineering techniques to increase enzyme activity [251,252].

In the work of Kupski et al . (2019) has been proposed to optimize the laccase -mediator system (LMS) to reduce pesticide levels in the aquatic environment [170]. Among all tested mediators, the best degradation efficiency was achieved for the laccase -vanillin system at 30°C after 5h incubation.

Laccase mediator system (LMS) can overcome obstacles such as steric hindrance (for macromolecular compounds), very low affinity between the compound and the active site of the enzyme, and high redox potential of putative substrates. ABTS is the first synthetic molecule to act as a laccase mediator [91].

To improve the stability of enzymes, increase their activity in a wide range of pH and temperature is being used immobilization. This process also allows the enzyme to be used multiple times and facilitates its storage, which reduces the cost of the reaction. Methods such as adsorption, entrapment, encapsulation, covalent bonding, and self-immobilization have been used to immobilize laccase[74,253–259].

In the Nair et al. (2013) study, *Coriolopsis gallica* laccase immobilized on mesoporous silica spheres by adsorption cross-linking has been used to eliminate endocrine disrupting compounds (EDCs) from wastewater [260]. Compared to the free enzyme, the immobilized enzyme had significantly higher thermostability , with half-lives of 31.5h and 3.9h respectively compared to 6.1h and 0.6h at 55°C and 75°C. The improvement in temperature resistance in the biocatalyst was probably due to the reduction of molecular mobility and the improvement of conformational stabilization due to the high degree of multi-point attachment to the substrate. Immobilized laccase used in a continuously stirred membrane reactor eliminated more than 95% of 10mM BPA and 10mM EE2 and 70% of 10mM diclofenac when treated individually and more than 90% when treated as a mixture in a pH 5 aqueous buffer solution for more than 60 reactor volume. After more than 80 hours of real wastewater treatment, over 85% of BPA and EE2 and 30% of diclofenac have been degraded.

Much attention is paid to nanomaterials and nano -biocatalysts. The nanomaterial particles form a complex with the enzyme through a covalent bond [261]. Nanomaterials provide a larger immobilization surface, which increases the enzyme load per unit mass of particles and thus increases the efficiency of immobilized enzymes [262,263]. The reuse of immobilized laccases improves the efficiency and durability of the process by reducing the costs associated with the loss of enzymes and materials [146,264].

To improve the decolorization performance of HR dyes Wehaidy et al (2019) developed a method of laccase immobilization on a nanoporous Zeolite-X carrier [265], chemically belonging to hydrated aluminosilicates, forming a three-dimensional network skeleton with the same size of interconnected pores and channels. Covalent immobilization on the nanomaterial improved enzyme properties such as resistance to pH and temperature changes, lowered activation energy and increased catalytic efficiency. Complete

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discoloration of AB 225 occurred after 15 minutes of incubation and RB 19 after 45 minutes. In addition, the immobilized formulation retained 100% of its original activity after 7 consecutive decolorization cycles. Laccase of *Polyporus durus* ATCC 26726 immobilized on nanoporous Zeolite-X (ZX) can be successfully used in the textile industry for water treatment.

Genetic engineering and protein engineering techniques are used to modify the thermostability, catalytic activity and substrate specificity of enzymes. Three types of enzyme modification strategies are used in this field, such as rational, semi-rational and directed evolution methods [72,140].

By site-directed mutagenesis of Xu Journal of Bioscience and Bioengineering 2020 obtained mutant S208G/F227A CotA-laccase Bacillus pumilus W3, which showed more than 5 times higher catalytic efficiency than laccase wild-type CotA and improved methyl red decolorizing capacity.

Based on the method of site-directed mutagenesis Xu et al. 2019 also investigated the role of N- glycosylation in specific laccase activity *Coprinopsis cinerea* Lcc9 expressed in *Pichia pastoris* [266]. It turned out that the glycosylation at N313 affects the affinity of the enzyme for substrates, and the glycosylation at N454 - the catalytic rate. Notably, N-glycosylation modifications in eukaryotic hosts can significantly improve the thermostability of lignocellulosic enzymes, as the glycan chains act as a protector by attaching to the protein surface via extensive hydrogen bonding [267]. Heterologically expressed fungal laccases exhibit different biochemical properties compared to native fungal laccases. Laccase *Cyathus bulleri* when expressed in *P. pastoris* , in which the glycosylation pattern was changed, increased thermal stability and salt tolerance, which is likely related to the change in protein structure and function.

Rational engineering increased the substrate specificity of the laccase lccb *T. versicolor* for the oxidation of large polycyclic aromatic hydrocarbons without mediator [171]. The authors used structure-based protein engineering to generate rationally modified laccases with enhanced ability to process large PAHs without a mediator. Computational simulations were used to estimate the effect of mutations in the enzymatic binding pocket on the binding and oxidizing capacity of a selected set of organic compounds. The generated mutants with an enlarged laccase binding pocket showed increased activity without the mediator (up to approximately 300% at pH 3.0) and functioned over a wider pH range (3.0-8.0) compared to the wild-type enzyme. Enlargement of the binding pocket allowed macromolecular substrates to come into direct contact with the catalytic residues of the laccase, which eliminated the use of mediators for electron transport. The modified laccase degraded ethyl green, a synthetic triphenylmethane dye, in over 90% within 24 hours without mediators. The researchers proposed the use of the M1 catalytic site mutant of the F162A/L164A laccase in mediator-free degradation of high molecular weight PAHs in bioremediation , wastewater treatment (e.g. textiles) or industrial processes (e.g. sulphate bleaching).

Non-catalytic enzyme functional sites are modified according to the primary and tertiary structure of the protein, while catalytic functional sites are designed semi-rationally and rationally based on molecular docking [268,269].

Using advanced molecular biology tools, i.e. next-generation sequencing, site-directed mutagenesis, fusion proteins, surface display, etc., scientists can now develop enzymes to improve activity, stability and substrate specificity to meet the stringent conditions of industrial processes and biodegradation of waste from their activities [251].

Currently, work is being done to modify the enzymes, which are then immobilized. When a charged polypeptide tail was added at the C-terminus, the salt resistance of laccase placed in coacervate core micelles (C3M) by encapsulation was enhanced [270]. The use of CotA with a polyglutamic acid tail resulted in more micelles with fewer enzyme molecules per micelle, improved salt stability of enzyme-containing C3Ms in the reaction medium, and increased enzyme activity. Increasing the net charge of enzymes by genetic engineering appears to be a promising strategy to improve the practical application of C3Ms as enzyme delivery systems.

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7.2. Improvement of laccase productivity

Laccases are produced by various groups of organisms from bacteria to plants. Biotechnologically important is the production by bacteria and fungi. The physiological requirements of microorganisms capable of producing laccases are different. To obtain the maximum amount of enzyme, the influence of many factors, such as the carbon source, concentration and type of nitrogen source, pH , temperature, inducer and other culture conditions on laccase production is studied [91].

The synthesis and secretion of laccase are strictly dependent on the level of nutrients, culture conditions, developmental stage, as well as the addition of a wide range of inducers to the culture media. Most of these factors have been shown to operate at the transcriptional level, producing different enzyme isoforms within the same strain and between different fungal species [269].

Inducer of laccase production in fungal cultures, in addition to high nitrogen content in the medium *Basidiomycete* I-62 (CECT 20197) and *Pleurotus sajor-caju*, is the presence of copper and other metal ion or aromatic compounds structurally similar to lignin precursors [91,181].

Optimizing fermentation is an important step in increasing laccase production , and promising results can be achieved by changing just a few parameters. This is the cheapest way to overproduce these biocatalysts. Therefore, each step of the fermentation process must be carefully optimized to obtain the desired enzyme expression levels. Modern methods more and more willingly used to optimize the composition of media is the methodology of the response surface (RSM) [252]. With good results, RSM was used to optimize the production of laccase by *Agaricus bisporus* CU13 [271]; *Pseudolagarobasidium acaciicola* AGST3 [272]; *Penicillium chrysogenum* [211].

Atilano-Camino et al. (2020) optimized the production of laccase from Trametes using the RSM method versicolor in batch submerged fermenters using lignocellulosic residues (agave pomace, coconut fibers and wheat bran) as co-substrates [273]. The results of the study showed that optimal conditions for the production of laccase were found at 35 °C and 5 g/l wheat bran as a co-substrate , reaching about 200 U/ml in 11 days in batch submerged fermentation. The supernatant was used in the enzymatic degradation of wastewater with the removal efficiency of CHOD at the level of 43% and the increase of the biodegradability index from 0.64 to 1.36. Overall, as noted by the authors, laccase- mediated enzymatic biodegradation may become a viable strategy for pretreatment of real wastewater.

Wild-type hosts can only produce small amounts of enzymes and are unable to meet industrial demands. Therefore, *P. pastoris*, *Aspergillus oryzae*, *A. niger*, *Aspergillus nidulans*, *T. reesei* and *Yarrowia lipolitica* are often used for heterologous expression of laccases [269].

Recently, Clark et al. (2021) used the insect *Drosophila melanogaster* for functional expression of a laccase from the fungus *Trametes trogii* [274] . Raw waste can be fed directly to insects without the pre-processing, refining and sterilization necessary for microbial fermentation. The short tubulin promoter was used to achieve moderate expression in many tissues of the insect, and the native fungal signal peptides were replaced with the *D. melanogaster* larval epidermal protein signal peptide to facilitate extracellular secretion. Insects expressing laccase degraded the BPA present in the medium in more than 50%. A lyophilized powder made from processed adult flies degraded over 90% of BPA and indigo carmine in aqueous solutions. The obtained results show that transgenic animals can be used for bioremediation environmental pollutants in vivo and serve as new production platforms for industrial enzymes.

To sum up, the aim of traditional methods of optimizing the production of laccases as well as methods of genetic engineering in heterologous expression of enzymes is to produce as many laccases as possible with appropriate biochemical properties at very low costs [91,269].

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8. Conclusions

The increasing population and industrialization result in the generation of large amounts of sewage containing substances dangerous to the health of humans and animals and disrupting the functioning of entire ecosystems. It is necessary to search for environmentally safe methods of removing xenobiotics from waters. Enzymatic degradation of pollutants using laccase is a good alternative to traditional wastewater treatment methods, as it does not generate harmful by-products. It is important to search for new sources of laccases, their biochemical characteristics, improvement of catalytic activity, thermostability and increase of productivity using modern methods of genetic and protein engineering. This will contribute to the improvement of biodegradation processes involving enzymes and will increase the efficiency of removing xenobiotics from the environment.

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