WHITE-ROT FUNGI AS POTENTIAL BIOREMEDIATORS OF ENDOCRINE DISRUPTING COMPOUNDS – A MINI REVIEW

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1. INTRODUCTION

Disruptive bioactive compounds are an emerging concern to human, animal, and environmental welfare. Such compounds are either synthetic or naturally occurring and have the potential to interfere with homeostasis, alter behaviour, or show toxicity in organisms. Examples of such compounds include insecticides such as DDT and clothianidin (neonicotinoids which are considered major causes for bee starvation [13]), industrial by-products like hydrogen cyanide (a toxin [15]), and aflatoxin B1 (a very potent carcinogen produced by the soil-borne fungi of the *Aspergillus* genus [16]). As indicated by the examples, disruptive bioactive compounds can be highly dangerous for human and animal health. Therefore, it is of importance that modes of action, toxicity, accumulative ability, and remediation of such compounds are well-known. Despite contemporary regulations regarding the use of bioactive compounds in industries, novel bioactive compounds that cannot be removed effectively are still being discovered globally. One category of such disruptive bioactive compounds is 'endocrine disruptive compounds'.

Currently, the most widely accepted definition of an endocrine disruptive compound is: "*a substance, either natural or synthetic, which, through environmental or inappropriate developmental exposures, alters the hormonal and homeostatic systems that enable the organism to communicate with and respond to its environment*" [7]. Briefly, endocrine disrupting compounds (EDCs) are compounds which are able to interfere with endocrine systems. An endocrine system is the collection of glands and receptors that are involved in hormonal production, regulation, and metabolism of hormones in organisms [17]. Endocrine systems are essential for the regulation of metabolism, development, sexual function etc. and are in this way responsible for normal, healthy life [17]. EDCs comprise a very diverse group of compounds, ranging from medicinal compounds to actual hormones, from metals to phthalates (common plasticizers e.g. used in PVC-plastics), [18, 19]. EDCs occur in many industries using EDCs and include pesticides, steroids, and constituents of common plastic [7]. EDCs are widely spread in the environment as they have been used and produced for decades [1]. One of the major sinks of EDCs is surface water and effluent streams [20, 21]. As water is one of the primary inputs for our food, many consumed products are subjected to these compounds, forming a biohazard to global health [22]. Their large-scale effects on human health and the environment have been leading to increased scientific interest in the degradation of EDCs. Bioremediation – the remediation of compounds by using microorganisms- might provide solutions for EDC removal. Especially so-called white-rot fungi (WRFs) seem to be effective due to their naturally occurring pathways that are able to degrade EDCs in an effective manner.

WRFs are a physiologically categorized group of fungi that are able to degrade lignin, a complex polymer present in woody, plant-based materials [23]. The term 'white-rot fungi' refers to the white, bleached appearance when the lignin has been degraded from woody materials [24]. WRF are obligate aerobes and comprise many basidiomycetes and few ascomycetes [24, 25]. Their lignin-degrading ability is owed to secreted enzymes and supplementary pathways. Enzymes and pathways involved in lignin-degradation have broad substrate specificity, allowing the catalyzation of reactions associated with other diverse substrates besides lignin, including a wide range of toxic compounds [26]. Especially the use of WRF for industrial dye effluent treatment has already been widely studied, showing high efficiencies and economic feasibility [27-29]. The same mechanisms used for lignin-degradation and dye effluent treatments can be used for the bioremediation of EDCs [30] [31] [32].

This review will focus on mycoremediation; the use of fungi to perform bioremediation. More specifically, the review will be tailored towards the use of WRF to bioremediate EDCs present in surface water and effluent streams that cannot be effectively removed by current water treatment plants. The aim of this review is to give a comprehensive overview of recent developments and prospects in the field of WRF-mediated remediation of water abundant EDCs. To this end, first, an overview of the issues related to EDCs and their impact will be provided. Afterwards, contemporary EDC-related mycoremediative mechanisms of WRFs will be discussed. Subsequently, water systems in which EDCs are removed are elaborated upon and assessed. Finally, knowledge gaps and future perspectives are discussed to allow for improvement in the remediation of EDCs by WRFs and to stimulate and steer research within this field.

2. OVERVIEW AND IMPORTANCE OF ENDOCRINE DISRUPTING COMPOUNDS

The following paragraphs elucidate on the types and occurrence of EDCs. Furthermore, the impact and importance, and complexity of the problems associated with EDC activity are discussed.

2.1 Types and sources of EDCs

In general, EDCs are all around us. Many industrial products and by-products contain EDCs. Moreover, EDCs are present in products we use for daily consumption such as fragrances, pharmaceuticals, and personal care products (Fig. 1) [33]. Many compounds that have endocrine disruptive effects are still being discovered in our day-to-day products. To exemplify, until recently, many plastic bottles and billing receipts contained Bisphenol A (BPA), contributing to almost daily intake or exposure of this compound in / to humans. Now it is considered a model compound for EDCs and strict regulations are in place. EDCs can be categorized in various ways. In literature the most common classifications are based on chemical properties, source [33] and use [34] [35], or synthetic versus naturally occurring compounds (Fig. 1) [7].

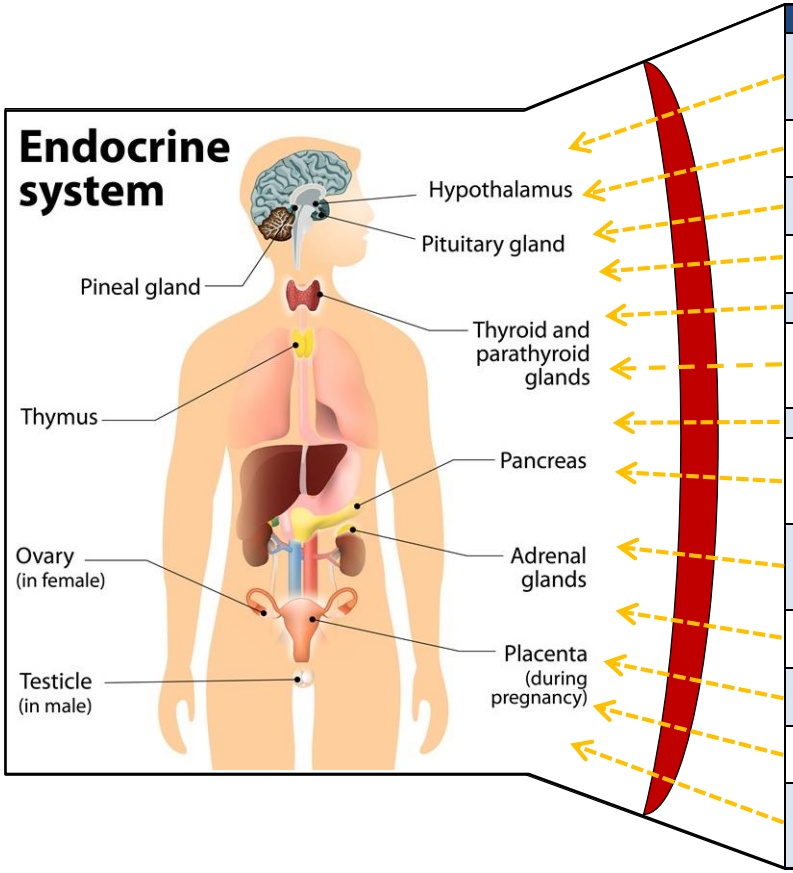
Major sources of EDCs include industry and agriculture (Fig. 1) [36]. In industries, EDCs are often formed as by-products or suboptimal conversions (during incomplete combustion). Examples industrial EDCs include alkyl phenols (found in coatings, detergents, and fuels), heavy metals (from mostly mining industries), and dioxins (resulting from recycling or waste industries). Most industrial EDCs end up in industrial effluent waste streams, which in turn leads to surface water [37, 38]. Agriculture associated EDCs include crop protection products that contain bioactive compounds (biocides: insecticides, herbicides, pesticides) and hormones used in livestock [39] [40]. Farmers spray biocides on their fields, allowing EDCs to spread through the air. Moreover, biocides that did not target the crops may stably reside on the field / in the ground. Similarly, livestock originated EDCs are excreted through urine and faeces and remain in agricultural fields / ground environments. Ultimately, agricultural EDCs end up in surface water due to run-off processes [36, 37, 41]. As the major EDC-producing sources –among which industry and agriculture- eventually lead to surface water, surface water is considered to be a major sink of EDCs worldwide [20, 21] [36, 37, 41, 42].

2.2 Impacts and complexity of endocrine disruptive compounds

2.2.1 Endocrine disruptive effects

The main reason why EDCs are considered an important issue is due to downstream effects of the substances. EDCs lead to a plethora of aberrant effects observed in human and animal health and the environment [7]. Many EDCs cause such effects in even minimal trace concentrations [43]. Endocrine disruptive activity by EDCs in humans is caused by exposure to the environment, or through diet [44]. EDCs are associated with an array of diseases in all organs of the human endocrine system. EDCs have been shown to negatively affect male and female reproduction, breast development, neurological functioning, metabolism, cardiovascular health, and stimulate various forms of cancer and obesity [44]. Moreover, EDCs have been shown to affect the epigenome. In this manner, the undesirable effects of EDCs are observed to span multiple generations, as epigenetic markers are inheritable (reviewed by Skinner et al., (2011)) [45]. To illustrate the scale of the EDC problem, current conserved estimations of the burden of EDCs in human health are in the range of at least 100 billion euros [46].

Besides effects in human welfare, many EDCs are bioactive in animals and other wildlife introducing major health issues. Effects associated with endocrine disruptive activity include reduction in fertility, changes in immunological systems, and reduction in progeny fitness [10]. In extreme cases, EDCs have been reported to cause gender switches within populations, consequently leading to aberrancies on an ecological scale, most prominently visible in amphibians [40]. Aquatic ecosystems are especially threatened by EDCs, as EDCs accumulate in waters to high concentrations [42].



Type of EDC	Examples of compounds	Use / source
Alkylphenols	Nonylphenol, propylphenol, butylphenol.	Precursors for detergents, fuels, fragrances, adhesives
Bisphenols	Bisphenol A, Bisphenol S	Used in plastic industry, flame retardants
Brominated flame retardants	Hexabromocyclododecane, tetrabromobisphenol A	Used in textiles, furniture
Dioxins, furans	2,3,7,8-tetrachlorodibenzo-p-dioxin, and 2,3,7,8-tetrachlorodibenzofuran	By-products of burning waste, tobacco smoke
Heavy metals	Cadmium, mercury, lead	Industrial mining
Hormones	Estrone, 17β-estradiol, estriol, diethylstilbestrol	Contraceptives, steroids, secretion by human and animal urine
Organotins	Tributyltin chloride, bis(triphenyltin) oxide	Paints, plastic stabilizers
(Organochlorine) pesticides	DDT, DDE, atrazine, tributylin	Agriculture, to combat plagues, diseases, weeds
Parabens	Methylparaben, ethylparaben, propylparaben	Preservatives, cosmetics, personal care products
Phthalates	Di (2-ethyl-hexyl)-phthalate, diisobuthyl-pthalate, di-n-buthyl-phthalate	By-products plastic industry, detergents
Phytoestrogens	Genistein, daidzein	Naturally occurring plant compounds
Polychlorinated biphenyls (PCBs)	2,2 ,4,4 -Tetrabrominated diphenyl ether, 2,5-dichloro-4, hydroxybiphenyl	Coolants, lubricants (no longer in use).
Polycyclic aromatic hydrocarbons (PAHs)	Naphthalene, fluorene, anthracene	Products of incomplete combustion of organic material

Fig. 1: Schematic overview of the human endocrine system and EDCs (incl. types, examples, and sources) that can interfere
Hormones, imply both naturally occurring and synthetic hormones that can have endocrine disrupting effects [1]. The uses and sources indicate that EDCs are implemented in products that we use on a daily basis. The EDC overview has been adapted from [7] and [10] and has been complemented with additional information.

2.2.2 Complexity of screening and control of endocrine disruptive compounds

The physiological and mechanistic diversity of EDCs hamper EDC-screening and monitoring which makes control of EDCs and its effects difficult. It is currently not possible to indicate whether a compound has endocrine disrupting function based on its structure as EDCs can have many physiological properties [44]. In addition, modes of action also differ per compound; EDCs can affect endocrine systems in various ways: stimulating, inhibiting, or blocking. Moreover, they can do so in many ways including binding to hormonal cellular receptors, influencing co-activators, inhibiting endogenous hormone synthesis, or influencing the expression of endocrine pathways by altering promoter functions [47]. As underlying mechanisms of EDCs differ extensively, standardised screening experiments are not always feasible. In addition, as observed with many toxic compounds, EDCs frequently show dosage effects, and effects of EDCs can be masked by natural feedback mechanisms, life stages of exposure etc. Besides, EDCs may only show effects during specific life stages [48].

In addition to diversity, persistence in the environment and animals is another key concerning aspect of EDCs. Although EDC stability varies amongst the different EDCs, some highly active EDCs may continue to persist in the environment for years [49]. Such stable EDCs are considered most worrisome as they are most able to spread widely throughout the environment by air (outside [50] and inside homes [51]), water (open [52] and drinking water sources [36]), or by residing in the ground (especially land in agricultural use [53]). Many endocrine disruptive compounds are still abundant despite having been prohibited for decades due to proven toxic effects. For example, the banned pesticide DDT was banned in 1972, yet was frequently found in people's homes of U.S. citizens three decades later [51]. Bioaccumulation of EDCs can lead to the build-up of high concentrations of EDCs in animals, introducing the risk of animal disease and the presence of dangerous EDCs in human food [54]. The persistence contributes to another increasing concern regarding mixtures of EDCs: many stable EDCs occur in environments simultaneously. Although EDCs are often not toxicologically tested in admixed conditions, studying the risk of mixtures of EDCs is important as EDCs have the risk of amplifying each other's function in such conditions, which is called toxicity synergism (Box 1)[14, 51]. Besides, numerous compounds causing endocrine disruptive effects do not necessarily have the endocrine disruptive ability individually, but instead only potentiate endocrine disruptive action when present with other compounds. Testing mixtures of individual compounds without known EDC-related function is a non-targeted process, making it time-consuming. Overall, studies show that mixtures of compounds are increasingly relevant in the environment and human health (Box 1).

Altogether, the diversity (variation in dosage-effects, poor predictability and complex modes of action), and persistence of EDCs are the reason why EDCs are still being discovered to date, and why EDCs are considered an emerging problem.

Box 1: Glyphosate: the importance of testing mixtures of compounds in toxicity research

Glyphosate was recently widely covered by the media. Glyphosate, also known as Roundup® is a well-known herbicide, affiliated with the company Monsanto. Glyphosate is an infamous product, as many claims suggested the herbicide to have carcinogenic [4], teratogenic [8] and endocrine disruptive effects [4]. The active ingredient of glyphosate was – despite the claims – proven to have a low toxicity to human cells, considering the applied concentrations and possible exposures. Yet, this year novel research by Rice et al., (2018) elucidated that the chemicals mixture formulated in Round-up (including the glyphosate) is much more toxic and disruptive than the individual active ingredient [12]. The latter shows the importance of EDC screening of mixed substances rather than individual components due to potential toxicity synergism [14].

3. MYCOREMEDIATION OF ENDOCRINE DISRUPTING COMPOUNDS BY WHITE-ROT FUNGI

WRFs have the ability to degrade organic EDCs because of their unique set of broad acting, lignin modifying enzymes (Table 1). Moreover, WRFs have complementary pathways that enhance the breakdown of EDCs. In the following paragraphs, the mechanisms of EDC degradation by WRFs will be discussed, and overviews will be given of species, enzymes, and systems showing EDC-mycoremediative ability.

3.1 Lignin modifying enzymes of White-Rot Fungi

WRFs are known for their ligninolytic ability. The mechanisms used for ligninolysis are however also suitable for the degradation of organic endocrine disruptive compounds. WRFs have an array of extracellular, broad-acting lignin modifying enzymes (LMEs) [24]. The main LMEs of WRFs are oxidases and act on EDCs as substrates. The term oxidase refers to catalysing oxidation/redox reactions. Oxidation reactions are chemical reactions in which oxidation states of atoms are changed by means of electron transfer [55]. In the process of oxidation of the substrate, various free radicle reactions follow, depending on the substrate and environmental conditions [56]. Commonly, coupled (dimerized/polymerized) free radicles form or oxidative carboxylation reactions take place [55]. With a single EDC as substrate and purified LMEs of WRF origin, resulting product compounds range from high to low molecular weight compared to the substrate. The most abundant products resulting from LME catalytic activity are polymerized substrates [30, 56]. The general consensus is that the polymerization of endocrine disruptive compounds by LMEs are directly associated with a decrease in endocrine disruptive activity [30]. The latter has been shown for multiple EDCs among which the model EDC compounds bisphenol A (BPA), nonylphenol (NP) and triclosan (TCS) [30]. The main LMEs associated with EDC-degradation are laccases and peroxidases. Moreover, WRFs have additional enzymes and pathways available to enhance LME functioning [24, 57, 58]. These enzymes and pathways are elaborated upon in the following paragraphs.

3.1.1 Laccases

Laccases, also known as polyphenol oxidases, (classified as EC 1.10.3.2) are one of the earliest discovered enzymes [59] and have a large number of biotechnological applications ranging from the degradation of xenobiotics, to biosensors, and food preservation [60, 61]. Due to the very broad substrate range and diversity of laccases, a true definition of laccases is difficult [62]. Next to WRF laccase diversity inter-species, the same species of WRF have been shown to produce laccases with different enzymatic properties, called isozymes (or isoenzymes / isoforms of enzymes). Natively, laccases have been found to be essential in lignin-degradation for WRF [63] in which they act on the products (mainly phenolic compounds) that are released in the process of lignin breakdown by other enzymes such as peroxidases [64]. WRF laccases are glycoproteins and are secreted extracellularly [62, 65]. Laccases from WRF origin are generally between 50 and 80 kDa large, have pH optima ranging from 3 – 5.5 and have temperature optima in the range of 30 – 75°C [66, 67]. One distinguishing characteristic of laccases relative to peroxidases is that laccases use oxygen (O_2) as the primary electron donor, and the oxidative by-product is water (H_2O). Laccases are therefore considered true 'green' catalysers [56, 66]. Laccases generally consist of two active sites, three domains, and four sites containing copper [61, 68]. The copper atoms play an important role in the enzymatic function of laccases since they function as electron donors of the laccase substrate, which is needed for successful oxidation [67]. The copper sites are a major factor determining the redox potential (oxidative potential)) and hence determine the specificity, efficiency and (indirectly) the biotechnological interest of the enzyme [69]. Redox potentials of WRF laccases are found between 0.34V and 0.8V, which is the lowest redox potential from the WRF-derived LMEs. The factors determining the redox potential of the laccases are very complex and remain not yet fully understood [56, 70].

An in-depth overview of characterised laccases of fungi is given in Baldrian (2006) which shows that most ligninolytic fungal species produce at least one laccase isozyme [62]. Nevertheless, laccases of WRF are not yet well characterized; the Protein Data Bank database contains very few characterized structures of WRF laccases. An overview of WRF laccases shown to have EDC degrading ability is

given in Table 2. So far, WRF laccases have been found to transform diverse EDCs containing phenolic and aromatic amine groups including alkylphenols, bisphenols, parabens, phthalates, PCBs, hormones, organochlorine pesticides [62, 71-73].

3.1.2 Peroxidases

Next to laccases, peroxidases are the other primary enzymes of WRF that are able to degrade lignin. WRF peroxidases are similar to laccases in the characteristics of having an aspecific nature and an oxidation mechanism. However, the reactions that are catalysed and the co-substrates are different.

Peroxidases (EC 1.11.x) are enzymes that catalyse reactions in the presence of peroxides (R-O-O-R). WRFs contain three major sub-classes of peroxidases with EDC-remediative properties: lignin peroxidases (LiPs: EC 1.11.1.14), manganese peroxidases (MnPs: EC 1.11.1.13), and so-called versatile peroxidases (VPs: EC 1.11.1.16) [24]. Like laccases, peroxidase types are all expressed extracellularly and rely on oxidative radical production leading to the transformation of endocrine disruptive compounds. However, instead of using oxygen as co-substrate, hydrogen peroxide (H_2O_2) is used. LiPs, MnPs and VPs are all expressed extracellularly.

LiPs of WRF are monomeric proteins with an average molecular mass of approximately 40kDa. The exact function and mechanism of WRF LiPs in lignin degradation remains unclear [74]. The general consensus is that LiP is not essential for lignin degradation, as many WRF have not been shown to express or contain genes for LiPs. Instead of copper (as seen for laccases), LiPs contain iron (Fe) ions as oxidising donors [75]. LiPs contain high redox potentials (up to 1.4V) [76], allowing oxidisation of phenolics, but also aromatic and non-phenolic compounds [76, 77]. Few studies have focused on EDC-degrading activity by WRF LiPs individually, but positive results have been shown for bisphenols and hormones so far [78]. Preliminary results show that LiPs –like laccases- decrease estrogenic activity by means of oxidation-based polymerization of EDCs [78]. Besides polymerization, LiPs have been reported to lead to radical production which in turn carry out reactions such as C-C bond cleavage, and hydroxylations, depending on the presence of mediatory compounds (compounds which enhance enzymatic function) and substrate [24, 78].

WRF's MnPs are compared to lignin peroxidases more widely studied, including their EDC-degradative capacity [30]. MnPs are very common enzymes among WRFs: many WRFs have at least one gene encoding for an MnP [79]. An extensive overview of manganese peroxidases found in WRFs and soil litter decomposing fungi is given in Hofrichter (2002) [79]. MnPs have an average molecular size of approximately 43 kDa [79]. Although MnPs are very similar the LiPs, manganese peroxidases are – as the term implies - generally dependent on the presence of manganese (with a few exceptions) as the preferred substrate relative to iron. MnPs are known to be capable of transforming phenolic compounds. The substrate specificity can be extended by means of the addition of mediators. In this way, non-phenolics can also be transformed by MnPs. Successful remediation by MnPs has been shown for the following types of EDCs: phytoestrogens, steroids, bisphenols, and numerous PAHs [30, 80]. Based on limited literature, WRF MnPs are reported to remove EDC activity of EDC by polymerization *and* cleavage products rather than merely polymerization as seen for WRF laccases and LiPs [81].

WRF VPs are a poorly defined, hybrid class of enzymes that are similar to MnPs in their functioning but strongly resemble LiPs on a molecular structural basis [82]. Instead of iron ions, they use other metals for oxidisation. Moreover, VPs are able to induce electron long-distance electron transfer. Out of all peroxidase classes, VPs are the most variable in substrates and redox potentials. This variability is the cause of the high potential of VPs in biotechnological applications [82]. However, despite papers emphasizing their biotechnological potential [83], the application of EDC-degradation has been barely touched upon. VPs are by far the least studied LME from WRFs. Only in recent years, EDC-remediation capacity of VPs has been shown for several hormones, bisphenols, and alkyl phenols by a single group of scientists [84, 85].

Enzyme type	E.C. number	General reaction [82]	Effective against (EDC-type)*	Size in kDa	General temp. optima range	General pH optima range	General redox potential range	Natural / native mediators	Synthetic mediators
Laccase	1.10.3.2	4 benzenediol + O_2 = 4 benzosemiquinone + 2 H_2O	Alkylphenols Bisphenols, PAHs, organochlorine pesticides [68, 86]	50-80	20-80 [82]	2-10 [82]	500-800 mV [27]	Humic acid, syringaldehyde [68]	ABTS, HBT, 3-HAA, NHA, Triton-X, TEMPO [27] [25, 68]
Lignin Peroxidase (LiP)	1.11.1.14	dimethoxybenzaldehyde + 1-(3,4-dimethoxyphenyl)ethane1,2-diol + H_2O	Bispohenols, Hormones, PAHs, [78, 87]	~ 40	35-55 [82]	1-5 [82]	1450 mV [27]	Veratryl alcohol, 2-chloro-1,4-dimethoxybenzene [88]	-
Manganese Peroxidase (MnP)	1.11.1.13	$2Mn^{2+} + 2H+ + H_2O_2 = 2Mn^{3+} + 2H_2O$	Phytoestrogens, steroids, bisphenols, PAHs [30, 80]	~ 43	30-60 [82]	2.5-6.8 [82]	1510 mV [27]	Mn^{3+}, organic unsaturated fatty acids (malonate, oxalate, glutathione) [25]	Tween 80, HBT [89]
Versatile Peroxidase (VP)	1.11.1.16	donor + H_2O_2 = oxidized donor + 2H_2O	Hormones, bisphenols, alkylphenols [84, 90]	~38**	20-50** [85]	3-6* [83]	Unknown	Na-malonate and Mn^{2+} veratryl alcohol [90]	ABTS

Table 1: An overview lignin modifying enzymes of white rot fungi and their characteristics and mediators
*The enzymes are effective against these EDC-types, but are not restricted to the categories. The enzymatic EDC-degradative characterization of the WRF LMEs is far from complete. The column indicates against which EDC-type the LME has been successfully tested.
**As versatile peroxidases of WRF are not well studied, the size and temperature optima range is estimated based on a very small sample size of studies. Redox potential could not at all be found in scientific literature at the moment of review. Preliminary studies show big range, spanning higher mVs than peroxidase LMEs [6]

3.2 Mediator enzymes

The enzymes types above are not the only types which enable ligninolysis and could have potential in EDC-degradation. Several enzymatic groups are considered auxiliary enzymes and aid by the production of co-factors or natural mediators that further increase the activity and / or specificity of the peroxidases and laccases. For example, enzymes such as glyoxal oxidase (EC 1.2.3.5) and superoxide dismutase (EC 1.15.1.1) produce H_2O_2 (co-substrate) for the functioning of LiP and MnP [27]. Additionally, they protect the fungi against the oxidative stress by superoxide compounds [24, 91]. Other beneficiary enzymes include glucose oxidases (EC 1.1.3.4) and aryl alcohol oxidases (EC 1.1.3.7) which have been found to cooperate with laccases [24]. In addition, they have been suggested to control polymerization of phenolic products and intermediates resulting from WRF laccase activity [92]. Other enzymes known to be involved in positively enhancing lignin degradative enzymes have been studied and characterized as well, such as oxalate decarboxylases (EC 4.1.1.2), formate dehydrogenases (EC 1.2.1.2), P450 monooxygenases (EC 1.14.x), and cellobiose dehydrogenases (EC 1.1.99.18) [25, 79, 93].

Although these mediator enzymes are known to boost the efficacy of LMEs and or its pathways, barely any EDC-remediative essays including WRF mediatory enzymes have been performed. Moreover, several novel oxidative mechanisms are still being discovered within WRFs, which might positively influence LME activity. In 2015 Westereng et al., discovered for the first time that WRFs express lytic polysaccharide monooxygenases (LPMOs: EC 1.14.99.54) that are also involved in long-distance electron transfer leading to polymerization of phenolic compounds during lignin degradation [94]. These LPMOs have not been tested for EDC-remediative potential yet.

3.3 Mediator and inhibitory compounds

LME and substrate specificity and activity can be further extended by means of using so-called mediator compounds, simply known as mediators. Mediators or ‘electron shuttles’ generally work by increasing the redox potential, allowing more substrates to be converted by oxidation reactions (broader specificity) and/or reach higher efficiencies [95]. E.g. laccases act on and transforming a broad range of phenolic compounds without any mediatory compounds, but in presence of mediators, laccases have the additional ability to extend their enzymatic activity to non-phenolic compounds. Alternatively, mediator compounds protect enzymes: veratryl alcohol was found to protect LiP against damaging effects of the co-substrate H_2O_2 [96].

Mediator compounds can be of synthetic or natural origin [95, 97]. The most widely studied and most effective synthetic mediators for the main LMEs are ABTS (2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) and TEMPO (2,2,6,6-tetramethylpiperidin-1-yl)oxyl), of which the latter is the most potent in most reactions [95]. High performing natural mediators include the compounds syringaldehyde, acetosyringone, and veratryl alcohol [97]. Both natural and synthetic mediators have the benefit of having optimal functioning in low mediator concentrations (low mediator/substrate ratio) [69]. Additionally, natural mediators compounds are easily and cheaply available from waste resources [98]. For an in-depth review of mechanistic functioning and an overview of synthetic and natural laccase mediators the paper of Cañas et al., (2010) is recommended [69]. Asgher et al., (2007) similarly describes mediatory compounds of WRF-derived MnPs [25]. A brief overview of mediators which enhance WRF LMEs in degrading EDCs is given in Table 1. In general, laccase-mediator interactions are the most widely studied. LiPs and VP-mediator interactions are not well described.

Several compounds inhibit or reduce the activity of EDC-remediating enzymes. They do so by binding to the active site of the enzyme and / or by decreasing the polarity, preventing the oxidative catalyzation of further reactions [25]. Examples include acetonitrite, dimethylsulfoxide (DMSO), n-propanol, azide, cyanide, cationic surfactant cetyltrimethylammonium bromide (CTAB), and several metals (Ag, Hg, Pb and Zn) [25, 99, 100]. Reversible inhibitors are also known: despite H_2O_2 being a co-substrate, it also has a reversible inhibiting effect on MnPs and LiPs in relatively high concentrations [79].

3.4 Potential of white-rot fungal enzymes for remediation of endocrine disruptive compounds

Approximately 10,000 species are categorized within the physiological group of the WRF [101]. Most Large-scale WRF screening assays showed that LMEs are present in most species that were found to be able to degrade lignin [102]. Kinnunen et al., (2017) found that from 53 fungal species, almost all WRF (96%) express manganese peroxidases and that 92% express laccases [5]. Overviews of currently known WRF LMEs are given in the reviews of Hattaka (1994)[103] and Kinnunen et al., (2017)[5]- providing a general overview of LMEs in WRF- Wesenberg et al., (2003)[27] -giving an overview of WRF LMEs with dye remediating capacity- Baldrian (2006) [62] and Morozova et al., (2007)[104] - reviewing the occurrence and characteristics of laccases in fungal species respectively.

Considering the scale and variety of LMEs found in WRF, studies focussing on EDC degradation by WRF LMEs are limited. My recent literature review focused on LMEs originating from WRFs with shown EDC-remediative capacity. The review yielded an overview of main LMEs (laccases, LiPs, MnPs, and VPs) and its species of origin. This overview is presented in Table 2. In total, 26 WRF species have been reported to express LMEs with EDC-degradative capacity. In addition, several species contain LMEs which have not been tested for EDC degradation. This is because many contemporary papers focus on merely one expressed enzyme of the WRF species, despite multiple LMEs being known. Altogether, when comparing reviews of WRF LMEs with Table 2, one can conclude that many detected enzymes with lignin modifying capacity have not yet been tested for their potential EDC-remediative potential.

WRF species	Laccases	LiP	MnP	VP	Additional remarks
Bjerkandera adusta	1 [105]	1 [32]	1 [32] [90]	1 [85, 90]	*Bjerkandera adusta* has been tested for EDC-remediative capacity. However, despite showing successfully mitigating the presence of the EDCs, novel undesirable endocrine disrupting compounds were produced, reducing the potential of these enzymes [32]. In contrast, a recent paper treating EDCs with isolated MnPs and VPs showed that endocrine effects were almost completely reduced [90].
Cerrena unicolor	1 [106] [107] [108]	DNT* [109]	DNT [110]	-	*C. unicolor* has a remarkably high production and secretion of laccase and MnP [111]. Eight laccase Isozymes have been detected for this species, at least one isozyme (or mixture) has been tested, unknown which one remediating activity [106, 107].
Coriolopsis gallica	1 [105]	DNT [109]	DNT [109]	-	Strain BCC 142 showed peculiar high expression of LMEs [109].
Coriolopsis polyzona	1 [30, 112, 113]	DNT [114]	DNT [114]	-	LME expression in C. polyzona is heavily affected by veratryl alcohol. The concentration of veratryl alcohol can completely repress LME production (at high concentrations), or stimulate 55-fold the amount of LME without the inducer [114].
Dichomitus squalens (Polyporus anceps)	1 [32]	-	1 [32, 115]	-	Two laccase and three MnP isozymes are discovered from this species, which isozymes were tested for EDC-remediative ability is unknown [116] [115].
Ganoderma lucidum	1 [117, 118]	1 [118]	1 [118]	-	*G. lucidum* was especially effective in degrading PAHs [117].
Irpex lacteus	1 [119]	1 [119]	1 [119, 120]	1 [32, 121]	Only traces of LiP activity were discovered. In total, three isozymes have been detected for this species [121]. In total, three MnPs are known for *I. lacteus*. It is unknown which isozyme(s) was / were tested [[121]. One of the MnPs shows remarkable stability and pH range (3.5-9) [122].
Myceliophtora thermophila	1 [123]	-	-	-	*M. thermophila* has not yet been classified / mentioned in literature as a WRF, despite being used for lignin degradative purposes. A special feature of the enzymes produced by *M. thermophila* is the thermostability: its enzymes have higher optimal temperatures than other WRF. Moreover, *M. thermophila* expresses several high potential thermophilic P450s and other auxiliary enzymes which could be of special interest as reviewed by Singh (2016)[124, 125].
Panus (Lentinus) tigrinus	1 [126]	-	1 [126]	-	Multiple laccase isozymes have been discovered, it is unknown which isozyme was purified for EDC-remediative ability [127].
Phanerochaete chrysosporium	DNT (unclear) [128]	1 [32, 87]	1 [32]	-	Whether or not *P. chysosporium* can produce laccases is unclear. According to sequencing studies, no laccase genes have been identified. In contrast, one laccase has been claimed to be purified form the species. 10 isozymes of LiP were identified by whole genome sequencing. Similarly, 5 isozymes of MnPs have been identified. [128, 129]. Indications of a VP gene expression was found additionally, but results should be verified [5].
Phanerochaete magnoliae	-	1 [32]	1 [32]	-	7 LiP isozymes were detected [130]. Which isozyme is detected and tested for EDC-remediative activity remains unclear. One paper from 1991 suggested inducible laccase activity, but it has never been confirmed with modern molecular tools [131].
Phanerochaete sordida	1 [132, 133]	DNT [134, 135]	1 [132] [133]	-	2 LiP isozymes have been detected but none have been tested for EDC-remediative capacity [134, 135].

Table 2: Overview of WRF containing at least one of the main LMEs (laccases, LiPs, MnPs and VPs) with shown EDC-remediative capacity
* DNT = Expression of enzymes have been Detected, yet Not Tested for EDC-remediative activity.
WRF species for which no proven LME with EDC-remediative capacity has been shown are excluded from the table.

WRF species	Laccases	LiP	MnP	VP	Additional remarks
Phlebia brevispora	1 [136, 137]	1 [136]	1 [136, 137]	-	Especially the PCB-remediative capacity of *P. brevispora*'s enzymes has been shown to be large [138].
Phlebia tremellosa	1 [9, 139]	DNT [140]	DNT [140]	-	The laccase of *P. tremellosa* has been purified and transformed into *I. lacteus*, which resulted in an 6 times higher laccase activity, with increased EDC removal rates [139].
Pleurotus eryngii	1 [141]	-	1 [141]	DNT [142]	Two laccase and manganese peroxidase isozymes have been detected in *P. eryngii*.[143, 144]. Laccase of *P. eryngii* was found remarkably effective in the removal of estriol compared to other WRF [145].
Pleurotus ostreatus	1 [32]	1 [146]	1 [32]	-	Recently, a novel biodegradation pathway was proposed, which could explain the unique conversion of *P. ostreatus* of phthalate compounds [147]. The latter could aid in understanding and exploiting this pathway for EDC-remediative purposes.
Pleurotus pulmonarius	1 [148]	-	DNT [5]	1 [5, 149]	*P. pulmonarius* expresses high levels of aryl-alcohol oxidase which increases the H_2O_2 level extracellularly [149].
Obba rivulosa (Physisporinus rivulosus)	1 [150, 151]	-	DNT [5]	-	Two laccase and two MnP isozymes have been detected [150, 152, 153]. Thermal optimum of laccases reached 50 degrees Celsius, and displayed extremely low pH optima of around 3.25 [154].
Pycnoporus cinnabarinus	1 [32, 123, 155]	-	1 [32]	DNT [5]	LiPs nor VPs have been detected in the fungal species' secretome [32]. Only recently VP expression (two types) was shown (in 2017) as VPs had remarkable expression induction: soy medium. The species' genome is known to contain a LiP encoding gene, but it's expression trigger is still unknown [5].
Pycnoporus sanguineus	2 [156]	-	-	-	Both laccases discovered showed high thermostability as well as high activity in cold conditions [156, 157]. The enzymes have been suggested to be of potential in harsh industries with variable environments.
Trametes hirsuta	1 [158]	1 [158]	DNT [159]	DNT [159]	Involvement of peroxidases and laccases has been suggested in the degradation of EDCs, but this still needs to be confirmed [158].
Trametes polyzona	1 [160, 161]	1 [161]	1 [161]	-	Strain RYNF13 was superior in most strains tested with highest removal rates and high thermostability [161].
Trametes pubescens	1 [162]	-	DNT [5]	DNT [5]	Two types of VPs were detected. Strain MUT 2400 secretes high efficiency EDC degrading laccase [162].
Trametes trogii	1 [163]	DNT [164]	1 [163]	-	LiP activity was detected on wood medium, artificial media were unsuccessful in triggering LiP [163]
Trametes versicolor	1 [165]	1 [32]	1 [165]	-	Multiple LiP isozymes exist considering a paper by Asgher et al., (2012) mentioning a novel LiP expressed by *T. versicolor* [166]. *T. versicolor* is a relatively the most widely studied species of WRF for EDC-remediative purposes [167].
Trametes villosa	1 [168]	-	DNT [169]	-	*T. villosa* expresses at least three laccase isozymes [170].

Table 2 (continued): Overview of WRF containing at least one of the main LMEs (laccases, LiPs, MnPs and VPs) with shown EDC-remediative capacity

* DNT = Expression of enzymes have been Detected, yet Not Tested for EDC-remediative activity.

WRF species for which no proven LME with EDC-remediative capacity has been shown are excluded from the table.

4. SYSTEMS TO REMOVE ENDOCRINE DISRUPTIVE COMPOUNDS IN WATER

4.1 Current water treatment solutions and their limitations

As previously mentioned, the majority of EDCs end up in water sources [42]. Water treatment is, therefore, a major focus of EDC remediation [171]. Contemporary water treatment plants are insufficiently effective in degrading EDCs. The main reason behind this is the limitations in the design of water treatment systems. Water treatment systems are designed for specific treatments against known contaminants. The latter specificity makes them not well adapted to novel, diverse identified and unidentified contaminants such as EDCs [171]. Other approaches are therefore required (Table 3). Most approaches make use of biological, chemical and nanotechnological mechanisms to remove EDCs from water. The most effective EDC treatments so far include reverse osmosis membranes, ultra- and nanofiltration, oxidation, activated carbon, and activated sludge [172]. An overview of what these approaches imply, including their advantages and disadvantages, is given in Table 3.

Overall, one of the biggest constraints of broad acting and effective treatment approaches is the high cost and the specificity. Approaches like reverse osmosis are heavily reliant on high-energy demanding processes. Maintenance costs such as the regeneration of granulated carbon filters or nanofilters furthermore hamper these approaches. Besides, waste streams that may are formed require further processing. EDCs are moreover notorious for passing through current approaches; they reject many of the treatments currently available. In literature, such compounds are often referred to as ‘rejected compounds’. The aforementioned aspects limit the widespread application of these methods.

Bioremediation is an approach which contrasts the hindrances of other approaches with regards to specificity and costs. Bioremediation generally does not require high energy input, which contributes to the cost-effectiveness and eco-friendliness of the approach [173]. Furthermore, bioremediation can complement and enhance pre-existing water treatment plants to degrade rejected compounds [173].

4.2 Systems using White-Rot Fungi to mycoremediate Endocrine Disruptive Compounds

Compounds that are rejected by current water treatment plants are mostly so-called trace organic compounds [174]. Trace organic compounds include EDC-types (organic pesticides, hormones, etc.) that are effectively degraded by the WRF mechanisms as described in chapter 3. WRFs additionally show high tolerances against toxic compounds and wide-ranged pH environments, which allows use in regular and toxic environments [25] [175]. The latter traits make WRFs quite adaptable for use in waste effluent streams which may contain toxins. Besides, an advantage of using WRF over other bioremediative organisms like bacteria is the presence of persistent antibiotics in wastewaters [176]. Bacteria cannot effectively handle such compounds, whereas WRFs are not negatively affected. Still, WRFs are in itself not enough to combat endocrine disruptors in the environment. The first systems have been designed and developed to implement and apply the theories of WRF-based EDC-remediation into practise and to maximize EDC-mycoremediative output. In the following paragraphs, an overview is given of such systems and their potential. In general, mycoremediative systems can be divided into two categories: systems with living mycoremediative fungi and systems in which isolated mechanisms of such fungi are exploited. Mycoremediative system conditions vary greatly between these two systems.

Type of EDC treatment	Brief summary	Advantages	Disadvantages	References
Granular activated carbon	A filter from carbon with sub-microscopic pores binding to organic matter	Effective against diverse organic compounds	Inorganic and hydrophilic compounds remain unaltered. Requires constant regeneration for effective functioning	[177-179]
Ultrafiltration and nanofiltration	Removal of particulate matter by forcing contaminated water through dense, small sized pores	Unspecific removal, depending on pore sizes removes bacteria and viruses	Less effective against dissolved (polar, hydrophilic) substances. Not economically feasible yet	[180, 181]
(Advanced) Oxidation	Use of free radicles to oxidize organic pollutants	Can enhance existing systems (both biological and chemical). Opportunity for technological optimisation	Currently no consensus on direction of optimisation. Intermediates can form which can be more harmful than parent compounds / original substrates. Energy consumption	[172, 179]
Reverse osmosis	Removal of particulate matter by forcing contaminated water a semi-permeable membrane	Filters better than nano-pores: final product is pure water without metal ions. Non-specific treatment	Rejection of certain compounds and mechanisms underlying rejection unknown. Reverse osmosis relies on a lot of power, financially constraining widespread use	[182]
Bioremediation	Using microorganisms to remove contaminants	Not energy demanding. Generally eco-friendly.	Research intensive investment required. Not a stand-alone approach. Monitoring required. Possible harmful intermediates	[183]
Activated sludge	Aerated sewage containing high concentrations of aerobic microorganisms breaking down organic matter (hybrid form of bioremediation)	Effective in removing carbonacaeous and nitrogenous matter and removing nutrients. Relatively energy effective (besides aeration requirement)	A wide range of pharmaceuticals reject the treatment. Results in waste sludge as by-product	[184]

Table 3: Brief overview of current, effective approaches to remediate EDCs in water
Although the approaches are individually presented, in water treatment plants, approaches are usually combined.

4.2.1 Systems containing living White-Rot Fungal cultures

Systems with living fungal cultures –also known as whole-cell cultures- have different requirements compared to enzymatic systems. For living fungal systems, it is necessary to stimulate the expression of mycoremediative pathways as promoters are stimulated by environmental responses [185]. If such pathways are not triggered, the expression of the enzymes drop, and accordingly removal rates are low [31]. Endocrine disruptor remediative pathway stimulation hence requires control of various inputs. One of such environmental triggers is the carbon / nitrogen (C/N ratio) balance in the environment to which WRF strongly respond. More specifically, high C/N ratios (often referred to as nitrogen starvation) increase expression [186]. Similarly, the presence of phenolic contaminants has a positive impact on the remediative enzyme synthesis [31]. As WRF are obligate aerobes, systems also require aeration [24]. Besides, moisture content, temperature and the pH of WRF cultures should be optimised on species level which implies finding the best conditions for fungal proliferation and enzymatic activity.

Due to the requirement of controlled environmental conditions, whole-cell systems are usually designed in the form of aqueous bioreactors to allow for continuous bioconversion of the substrates in wastewater. The most common bioreactors include fluidized bed reactors (FBRs), membrane bioreactors (MBRs), stirred tanks, bottle reactors and packed bed reactors [2]. The most efficient bioreactors make use of WRF cultures that are immobilized on matrices to prevent the spread of the fungus and minimize oxygen supply requirements [2]. An elaborate overview of various WRF bioreactor systems for wastewater treatment potential is given in a recent review by Mir-Tutusaus et al., (2018) and will hence not be discussed in this review [2].

So far, whole-cell WRF EDC-remediative system tests are limited to the degradation of only the model EDC-types such as BPA, nonylphenol, triclosan and several hormones. Besides, only *T. versicolor* and *P. ostreatus* have been tested in such systems [2]. The most efficient WRF whole-cell systems are highlighted in Table 4 to give an indication of the potential of whole-cell systems in EDC-degradation. Based on current, limited literature, results are very promising: purely taking the efficiency of WRF EDC-degradation into account, the general consensus in literature is that the whole-cell fungal systems have enough potential for real-world applications [2]. Whole-cell WRF systems allow for effective removal of EDCs in waters with EDC-concentrations reaching up to 20 mg/L (as tested for 17b-estradiol (E2)) which is considered extremely high (100 times higher) compared to real life wastewater situations [2] [187]. It should be noted, however, that the most successful tests are mostly done under sterile, controlled environments [188]. Sterility appears to have a large impact on the treatment duration for effective removal of EDCs. Non-sterile WWTP effluent requires multiple days for high EDC removal rates. Moreover, the papers describing these first ever whole-cell WRF systems describe that there are still several limitations that are holding whole-cell fungal systems back from industrial applications. Such limitations include:

1. Competition with native microorganisms: microorganisms use similar nutrients as the WRFs in the bioreactors, ultimately leading to increased pressure regarding the proliferation of fungi, and higher turnover rates of LMEs. The very few studies in continuously flowing non-sterile reactors (mimicking wastewater reactors) concluded that contamination of bacteria can be severely affecting the EDC-degradative efficiency [188]. Hence, tackling this issue is of primary, vital importance prior to up-scaling initiatives [188]. Solving this aspect by control of nutrients, and adding supplementary disinfectants is possible, such regulatory measures require further finances that will need to be taken into account.
2. Nutrients: although pilot studies on growth media worked well regarding the proliferation of fungal biomass, this aspect was found to be limiting in real wastewater treatment conditions. Literature is in consensus with the additional need for nitrogen and carbon in wastewater treatment settings [189]. Moreover, several nutrient balances (and other factors such as pH) lead to compromises between fungal proliferation and LME pathway activity [190]. Depending on the species, certain conditions favour the production on one type of LME over the other. For various taxa, LiP and MnP are optimally produced in high oxygen pressure environments. Contrastingly, laccase production is stimulated in submerged liquid cultures of WRF [27]. Similar to the nutrient balance aspect, compromises have to be made when designing systems and its cultures.

3. Treatment time: mycoremediation requires longer hydraulic retention time (HRT) compared to bacteria for high EDC-removal rates. Current wastewater treatment plants (WWTPs) are not well adapted for high HRTs required for mycoremediation, as current WWTPs treat high volumes of continuously flowing water. Reactors should therefore first be optimized to include either high concentrations of fungal biomass or batches of lower volume water flow. According to recent literature, EDC removal by means of WRFs can be made possible taking the latter recommendations into account [191, 192].
4. Ageing fungal biomass: like most organisms, WRFs become less potent over time in degrading EDCs. Hence, renewal / renovation of fungal biomass is to be considered. Although suggested in literature, WRF treatment pilot studies have not explicitly taken this aspect into account. For a continuous and autonomous system, new strategies have to be developed to combat the ageing process of WRF [193].

Endocrine Disruptive Compound	Fungal species	Treatment duration	Reactor type	Substrate	Sterility	Initial concentration	Removal (%)	Ref.
Bisphenol A (BPA)	*P. ostreatus*	28 d	trickle bed	WWTP effluent	No	20 ng/L	80	[191]
4-*n*-nonylphenol	*P. ostreatus*	28 d	trickle bed	WWTP effluent	No	10 ng/L	50	[191]
17β-estradiol (E2)	*T. versicolor*	26 d	FBR	Defined medium	Yes	3–18.8 mg/L	>99	[194]
17α-ethynyl-estradiol (EE2)	*T. versicolor*	26 d	FBR	Defined medium	Yes	7.3 mg/L	>97	[194]
	T. versicolor	110 d	MBR	Malt extract-based	No	5 µg/L	90	[195]
Estrone (E1)	*T. versicolor*	12 h	bottle reactor	WWTP effluent	Yes	350 µg/L	83.5	[192]
	P. ostreatus	28 d	trickle bed	WWTP effluent	No	45 ng/L	>99	[191]

Table 4: Overview of whole-cell WRF containing systems with EDC-degrading capacity
The systems in the table are a selection of the high potential whole-cell systems.
Adapted from Mir-Tutusaus et al., (2018) [2].

4.2.2 Systems based on isolated White-Rot Fungal enzymes

Input related factors such as C/N balance and the presence of phenolics are aspects that are not as important in systems in which isolated mechanisms of fungal species are exploited. The two main types of such systems include free and immobilized enzymatic bioreactors. Both systems imply bioreactors that rely on often high concentrations of WRF-derived isolated enzymes that convert the EDCs and in this process mitigate the endocrine disruptive effect. Accordingly, bioreactor conditions can be adapted for enzymatic functioning only, which is less multi-facetted compared to the adaption of environmental factors on the scale of the (developing) fungi. are optimized for substrate conversion with an emphasis on the enzyme rather than on stimulation of the organism.

The first type of enzymatic systems is called a free-enzymatic system. As the term implies, free enzymatic systems are systems in which the enzyme of interest is not attached to immobilized carrier media, but instead flows freely through the bioreactor. The consensus is that this type of enzymatic system is far weaker than the second type and will hence not be discussed in detail [71, 113, 196].

The second type is the immobilized enzymatic system. In this system, the enzymes of interest are fixed on carriers, which generally happens with high density. For this, high concentrations of enzyme are needed. Bioreactors with high concentrations of enzymes are obtained through in two general steps; high throughput enzyme production, and enzyme processing (isolation and immobilization). High throughput enzyme production is usually done in large-scale bioreactors that are optimized to induce high concentrations of specific LMEs. Laccases are for example often produced in stirred-bioreactors [197]. Subsequently, enzymes are isolated from the WRF cultures. Since LMEs are expressed extracellularly in liquid mixtures, relatively simple separation of fungal biomass and extracellular secretome can take place: centrifuging [113]. Afterwards, enzymes can be precipitated using e.g.: ammonium sulphate [113, 198]. Finally, enzymes require immobilization. Immobilization implies the attachment / fixation to inert material. Immobilization is useful for the following reasons:

1. It allows enzymes to become more stable: the fixation of enzymes prevents conformational changes that occur in free enzymatic bioreactors [199]. Moreover, thermostability is amongst others greatly increased when enzymes are fixed onto specific matrices [25]. Likewise, pH and temperature optima have been reported to be enhanced relative to free enzymes [198]. Catalytic properties can also be stimulated through immobilization [198]. Altogether, fewer enzymes are required for the same output when comparing immobilized and free enzyme systems.
2. Consistent throughput: compared to free enzymes, immobilized enzymes are more consistent in their output, as the catalytic activity is not dependent on location over time.
3. High-density enzymatic capacity: several matrices have been developed that can harbour high-density enzymes that have their catalytic sites in the right orientation. Relative to free enzymes, such conformations allow increased affinities to the substrates, as high-density filters can be created.
4. Reusability: immobilization allows for efficient recovery of enzymes, which alleviates the financial investment in enzyme production.

Extensive reviews are written regarding the immobilization of enzymes are written by Sheldon (2007), Datta et al., (2012), and Garcia-Galan et al., (2011) [200-202]. The reviews elaborate on the methods, materials, shortcomings, and optimization of immobilization. Despite the many advantages of immobilized enzymes, immobilization protocols require cheaper protocols as immobilization cannot always justify the cost of enzyme production [203]. Nevertheless, the consensus of scientific literature is that immobilized enzymatic bioreactors are far superior to free enzymatic systems, wastewater systems that use WRF-derived LMEs are no exception [204]. An overview of literature describing immobilized enzymatic systems from WRF to degrade EDCs is given in Table 5.

Endocrine Disruptive Compound	Fungal species	Enzyme type	Immobilization type	Treatment duration	Initial concentration	Removal (%)	Ref.
BPA	*B. adusta*	VP	VP–GOD-CLEAs	10 minutes	10 mg/L	73.6	[85]
	T. versicolor	Laccase	polyamide 6/chitosan nanofibers	6 hours	50 µM	92	[205]
Nonylphenol	*B. adusta*	VP	VP–GOD-CLEAs	10 minutes	10 mg/L	59.6	[85]
Triclosan	*B. adusta*	VP	VP–GOD-CLEAs	10 minutes	10 mg/L	N/A*	[85]
Triclosan	*T. versicolor*	Laccase	Chitosan-conjugation	6 hours	5 mg/L	100	[196]
17b-estradiol (E2)	*B. adusta*	VP	VP–GOD-CLEAs	10 minutes	10 mg/L	72.5	[85]
17-ethinyl-estradiol (EE2)	*T. versicolor*	Laccase	polyamide 6/chitosan nanofibers	6 hours	50 µM	96	[205]

Table 5: Overview of WRF immobilized enzyme systems with EDC-degrading capacity
*N/A: The study was not able to successfully measure removal rate. Bioreactor types were not well defined in the papers. All studies are pilots and were performed under controlled environments and with sterile, nutrient-rich medium.

4.2.3 Comparing whole-cell with enzymatic systems

At this moment, WRF-based EDC-remediative system studies are dominated by pilots. Larger-scale pilot studies that simulate realistic wastewater treatments conditions are required for definite conclusions regarding the best WRF-based EDC-remediative system [2]. Enzymatic systems have not yet been tested in environmentally realistic (non-sterile, mixed contaminants) wastewater treatment conditions. Contrastingly, whole-cell systems have been tested positive in such conditions and are therefore better established for real applications to complement current wastewater treatment plants at the moment of review [188].

Yet, in terms of EDC-remediative capacity, enzymatic systems seem to have more potential. When comparing current whole-cell and enzymatic system literature (Table 4 and Table 5), one striking differentiating aspect is the time required for treatment whilst having similar concentrations of EDCs. Immobilized enzymatic systems are in this view far more capable compared to whole-cell systems. Enzymatic systems allow furthermore for better control of the substrate conversion [94, 200]. The cause lies within the set-up of the systems: in enzymatic bioreactors, the active enzymes are isolated / purified and controlled. In contrast, whole-cell cultures may express mixtures of enzymes which cannot be controlled as effectively. This difference between the two systems is an important factor considering that intermediate products can cause more endocrine disruptive damage than EDC-associated substrates [32].

By comparing literature of both systems it became clear that research shows several gaps that still need to be addressed to allow for further development of the systems. These gaps are elaborated upon in the next chapter.

5. GAPS IN CURRENT LITERATURE

As mycoremediation of EDCs is still a relatively novel field, several gaps became apparent after literature review. In the following paragraphs, several of these gaps and opportunities are elaborated upon with the purpose of inspiring future research.

5.1 System design for the remediation of endocrine disruptive compounds by White-Rot Fungi

5.1.1 Unaddressed requirements of successful mycoremediative systems

To allow successful, large-scale practical use of mycoremediation-based systems, several requirements and standards must be met. Many of these are general bioremediative requirements [24], but are nevertheless important to consider for future system design:

1. The system must have adequate conditions to allow for mycoremediative activity. This includes the addition of inputs and environmental factors and the exclusion of system inhibiting or disrupting compounds.
2. The mycoremediator present needs to have mycoremediative capacity; it must be able to effectively lower the concentration of the substrate(s) to comply with satisfactory concentration standards.
3. The substrate(s) must be bioavailable to the fungi or the mechanisms thereof.
4. Intermediate compounds produced in the transformative process should be safe at the levels they are generated.
5. The financial costs of the system must be lower or equal to pre-existing alternative systems which can target the same substrate. Alternatively, they should be able to complement limited existing systems in transformative ability so that both systems can be used.

Current literature of WRF-based EDC-removal still seems to be focused on providing the proof of concept of certain enzymes being able to convert EDCs (point 2 and 3 above). However, it would be recommendable to keep the other aspects in mind to progress the field. As mentioned earlier, piloting with realistic wastewater inputs will give more insight into the applicability of the studies, and the effect of inhibiting compounds in wastewater are now unknown (point 1). Besides, current studies acknowledge the importance of intermediates, yet frequently do not provide the products generated by the WRF-derived enzymes of EDCs as substrates [32] (point 4). Finally, large-scale pilot studies in the future will hopefully provide an increased understanding of the cost-effectiveness. At the moment, cost-effectiveness is not yet mentioned sufficiently to be able to assess the economic feasibility of the systems (point 5).

5.1.2 Mediator compound implementation

Mediators are well-known to improve enzymatic functioning of WRF-derived LMEs [95, 97]. Nonetheless, current studies on EDC-capacity by WRF LMEs are still often performed without any mediator compounds. One potential argument why researchers leave mediator compounds out might be that mediators require additional costs. However, natural mediator compounds are easily and cheaply available from waste resources [98]. Considering the latter, complementing tests of EDC-remediative WRF-based systems with natural mediators is recommended, especially since they might further increase the systems' efficiency and cost-effectiveness.

5.1.3 Multi-enzyme cascade reactors

In scientific literature of enzymatic systems, single LMEs are often isolated and immobilized, after which they are tested in bioreactors. In such environments, substrate conversion and resulting product output are consistent. In nature LMEs and mediatory enzymes of WRF act in concert to effectively degrade lignin, contaminants and toxins. Prospective enzymatic systems could co-immobilize multiple types of enzymes in sequence [94]. Such architecture would allow for the catalysation of a cascade of biotransformatory reactions, whilst retaining controlled output of products and minimizing the risk of producing harmful intermediatory compounds [200]. An alternative to sequential architecture would be to cross-link of multiple enzymes, which is also described for WRF-derived LMEs [206].

5.1.4 Immobilization in whole-cell systems

Cabana et al., (2007) concluded that the competition with other microorganisms in whole-cell systems is the main hindrance of the approach [30]. However, immobilization, as described for enzymatic systems, may offer a potential remedy. Ehlers et al., (2005) proposed and tested immobilization of WRF on pellets containing lignocellulose. Microorganisms that are native to wastewaters seemed to have low affinity to such medium, and lignocellulose allowed the uptake of sufficient nitrogen and carbon for the proliferation of the WRFs (point 1 and 2 of the main limitations of WRF-whole-cell systems (4.2.1))[207]. Moreover, lignocellulose matrices can be cheaply made, as lignocellulosic material is a waste product of several industries [2, 82]. Finally, lignocellulosic matrices can be exchanged, allowing for the renewal of fungal biomass (point 4 of the same chapter). Despite this finding, further development of immobilization of lignocellulose-containing material remained absent. It might be worthwhile improve on the setup described in Ehlers et al., (2005) or discover similar materials which can cheaply address the major limitations of whole-cell cultures.

5.1.5 Mixed culture systems and complementation of LME-pathways

Mixed culture systems of White Rot Fungi are also very underrepresented in literature. Hai et al., (2012) showed that mixed bacterial-WRF fungal systems are more effective in the remediation of pesticides than bacterial and living fungal systems individually. The study showed that (unidentified) bacteria native to Japanese sludge waters increased pesticide-degradative efficiency and stimulated fungal enzyme secretion and that such cultures can be beneficial in aqueous systems.

Finally, besides engineering pathways from WRF, alternative pathways of non-ligninolytic fungi also have potential to increase the feasibility of EDC mycoremediation. In 2010, Różalska et al., proposed and described a novel pathway in *Gliocephalotrichum simplex* for degrading nonylphenols [208]. Studying such novel mechanisms could aid and / or complement knowledge in more widely studied WRF-associated mycoremediative pathways. Ultimately, the most effective mechanisms can be compared and engineered for true pilot studies.

5.2 Unexplored enzymatic potential

5.2.1 White-Rot Fungal Lignin Modifying Enzymes

Despite efforts to discover enzymes from WRF with EDC-remediative purposes, there are still many fungal species that have not yet been screened, and enzymes that have not yet been characterized nor tested. White-rot fungi are -despite their shared physiological function in lignin degradation- variable species. Each species has their own environmental preferences and optima. Accordingly, enzymes of various species vary in traits such as catalytic activity, thermostability, and substrate efficiency. It would thus be recommendable to explore the potential of WRF LMEs that have not yet been tested, for the purpose of finding EDC-remediative enzymes with higher efficiencies and / or high biotechnological capacity (Box 2). Even within species (various strains), significant differences in LME enzyme expression and characteristics are found [130]. The latter indicates that screening multiple strains per species is recommendable.

Box 2: Untapped potential of WRF: the case of *Phlebia radiata*

Relatively many studies focus on the detection of LMEs of only fungi containing the classification of 'White-Rot Fungus' without applications. Hence, sometimes gaps appear in literature where high potential LMEs of WRF are overlooked and not used to screen for the potential that they have. An example of this is the case of *Phlebia radiata*. *P. radiata* is a species that expresses all main LMEs: laccases, MnP, LiPs, and two types of VPs (Mn-dependent and Mn-independent) [5]. In a study by Kinnunen et al., (2017) both a LiP isozyme and two VP isozymes of *P. radiata* (strain 79) had convincingly the most enzymatic potential out of in total 53 screened LME expressing WRFs. Eg. the Mn-induced VP expressed by *P. radiata* was more than 7 times more active than the second most active screened WRF originating LME. Yet, very few articles are published on *P. radiata* and none of them includes the use of its enzymes for EDC-remediative purposes. It is very likely that *P. radiata* has potential in this field, also considering that its enzymes have high homology with a WRF that is known for high EDC-remediative capacity – *Phlebia tremellosa* [9]. *P. radiata* has been reported to express three isozymes of LiP (of which the expression is highly dependent on the medium state (liquid or solid)), two isozymes of MnP, and two laccase isozymes which could be studied for this purpose [11].

Phlebia radiata, also known as the 'wrinkled crust' is known for its wrinkled, orange-pink fruit body. It grows flat (like a crust) on its substrate, which is usually decaying wood from hardwood and coniferous trees. Underneath its fruit body the white-rot associated enzymes are secreted for nutrient uptake of the fungus, which speeds up the wood decaying process [3].

Moreover, in current literature regarding WRF-based EDC-remediation, isozyme variants are not mentioned. This is remarkable considering that many of the species that have been tested for expressing LMEs with EDC-remediative potential express multiple isozymes. At this moment, papers do not report which isozymes or mixtures of isozymes have been tested for EDC-remediative capacity. As a result, it remains unknown whether so-called 'isolated LMEs' in literature are mixtures of multiple isozymes, or if they are single isozyme variants that have been tested (Table 2: Additional remarks). In some cases, researchers might ignore WRF species which are reported by earlier studies to have inefficient EDC-degrading LMEs. However, it may be that those earlier studies did not test the best EDC-remediative isozymes. Moreover, isozymes can provide valuable insight into the optimization of enzymes by comparing the amino acid changes. Hence, for future research, it would be recommendable to not only report the efficiency of the LME types but also report which isozymes are being tested.

In addition, enzymes that are considered complementary / mediatory for the main LMEs (laccases, MnPs, LiPs, and VPs) have recently been shown to be more important in the conversion of undesirable compounds than previously thought [78]. Especially the enzyme P450 (cytochrome P450

monooxygenase) is known to influence EDC-remediation in a significant manner. Marco-Urrea et al., (2008) found that P450 is involved in the first steps of oxidation of very persistent pharmaceutical compounds [93], whereas the main LMEs did not play a role in their conversion. Moreover, Hideyat et al ., (2018) showed that P450 plays an essential role in the initiation of PAH degradation [158]. The initial paradigm that the main LMEs (laccases, LiPs, MnPs, and VPs) are solely responsible for EDC-degradation does not uphold any longer. Attention to supporting, intracellularly expressed enzymes such as P450 could benefit EDC-mycoremediative studies and prospective system designs.

5.2.2 Enzymes from brown-rot, soft-rot fungi and other fungi

Next to WRF, several other types fungi are capable of breaking down complex wood-associated polymers (cellulose, lignin etc.), such as brown rot fungi and soft rot fungi [209]. These fungi have not been studied widely, which can be explained by earlier paradigms that considered such fungi to lack the ability to express LMEs [82]. Recently, several studies pointed out that fungi besides WRF do in fact transcribe active LMEs. Laccases with high biotechnological interest (thermostability) have been characterized that have a brown rot fungal origin [210]. Furthermore, the classification of brown-rot and white-rot fungi has recently been shown to be inadequate, as several white-rot fungi do not show the traits associated with 'white rot' and vice versa [209]. LMEs from all wood degrading fungi should be considered instead of merely from those labelled as white-rot fungi. Studies on wood-degrading rather than white rot associated fungi could yield enzymes with high EDC-mycoremediative capacity.

Additionally, apart from wood-rot fungi, several fungi that are not well characterized could still contain enzymes with EDC-remediative capacity. Recently Junghanns et al., described aquatic fungi (*Clavariopsis aquatica, Myrioconium sp.* strain *UHH 1-13-18-4,* and *Phoma sp. UHH 5-1-03*) that express laccases which degraded xenoestrogens effectively [211, 212]. Such aquatic fungi could be of interest since –as the name suggests- aquatic fungi are naturally better adapted to water environments. As such fungi are native to aquatic environments, they could potentially be highly suitable for water treatment plants or water containing bioreactors relative to wood-rot fungi. Nevertheless, besides the papers of Junghanns et al., (2005, 2009) little to no papers have been published about such species of potential interest or their according enzymes.

5.3 Biotechnological engineering and breeding

In addition to further screening of species and enzymes, there are many opportunities for research focused on the optimisation of the enzymes, pathways and species that we already know have EDC-remediative capacity to make EDC-mycoremediation a reality.

5.3.1 Enzymatic optimisation

An obvious step in the optimisation of the degradative conversion of EDCs and their effects would be the engineering of enzymes. Enzymes can be optimised for several properties that will enhance their function in bioreactors or on immobilised filters. Natural variation in enzymes (such as isozymes) can be used to study amino acids that have an influence on enzymatic characteristics. Enzyme optimisation can be focused on many different aspects besides kinetic activity. Within enzymatic optimisation, several aspects can be focussed upon [213] (Table 6).

The first results of enzymatic optimization are promising: Hildén et al., (2012) obtained a 50-fold higher laccase enzyme in *P. rivulosus* by mutated a single amino acid in a heterologous system [150]. Combining novel approaches such as directed / targeted evolution, laccase activity from an unclassified basidiomycete (Basidiomycete PM1 / CECT2971) gained a 34.000-fold effective activity increase [214].

Enzymatic trait for optimisation	Explanation
Kinetic activity	Enhancing kinetic activity can boost the degradation of EDCs by raising the conversion rate of the substrate (EDC-associated compounds).
Tuning enzymatic optima	Enhancing enzymes for specific temperatures, pH values for use at *in-situ* bioreactors with specific environmental conditions.
(Thermo)stability	The turnover rate of enzymes is highly dependent on temperature. Improving enzymes to turnover less rapidly in higher temperature environments would assure overall stability. Enzymes which can be used longer (better activity over time) are preferred for remediative applications as it makes them more cost-effective.
Substrate specificity	Ensuring that the enzymes will bind to EDCs is of importance for optimal EDC-remediative applications. Binding of inhibitory compounds that reduce efficiency or block active sites is undesirable. Engineering enzymes with enhanced EDC affinity, and reduced affinity for inhibitory compounds is therefore recommended.
Allosteric control	Allosteric control is the regulation of enzymes by physical binding of a molecule besides the active site. Excluding such regulation is desired, as enzymes should not be downregulated in bioreactors.
Isolation potential	Being able to isolate EDC-remediative enzymes with high purity is desired to make EDC-remediation effective and financially feasible. E.g. specific isolative tags could be engineered onto enzymes.
Immobilisation potential	Immobilisation enhances stability, and increases the application potential for mycoremediation. Natural enzymes can be optimised for immobilisation by adding tags for increased stability and to ensure correct orientation of the enzymes' active sites.
Product control	Many WRF LMEs result in various products when converting substrates. It is recommendable to prevent intermediary compounds containing EDC-associated effects.

Table 6: Enzymatic properties that can be optimized for EDC-remediation with according explanations

5.3.2 Pathway optimisation

Molecular pathways can also be optimised to increase the biosynthesis, secretion of the EDC-remediative enzymes and / or enhance the activity of the enzymes. Optimizing pathways that enhance EDC-remediative capacity could lower costs of EDC-remediative enzyme production, and increase the efficiency of current EDC-remediative systems.

Several regulatory pathways have been discovered that influence WRF EDC-remediative ability. Such regulation includes –amongst others- the carbon/nitrogen balance (C/N) within the growth medium and the presence and concentration of phenolic compounds [31, 186]. During nitrogen starvation (high C/N ratios) and with high presence of phenolic compounds, several species have been shown to increase expression of pathways with EDC-remediative effects [31]. Light (daytime length) and temperature also seem to be of influence on EDC-associated pathways [185]. Additionally, a direct relation was found between the concentration of endocrine disrupting contaminants and the expression and release of EDC-remediative enzymes in *T. versicolor* and *P. chrysosporium* [215, 216].

The understanding of the molecular basis behind the regulation of LME pathways has been reviewed by Janusz et al., (2013) [185], but it is clear that many aspects remain unknown and more complex than previously thought. The regulation of LME production by means of cAMP control within cells is of special interest [185]. Several cAMP responsive elements have been found in upstream regions of promoters that regulate all main LME encoding genes in *Trametes versicolor* [185]. Moreover, several elements have been found that respond to N-starvation and various wavelength of light [185, 217]. Molecular studies exploring the regulatory mechanisms driving EDC-remediative pathway expression could be very beneficial to making mycoremediation more effective, by pinpointing molecular bottlenecks in such mycoremediation associated pathways [185]. Pathway optimization approaches have already been proven successful for dye decolourization purposes: overexpression of LMEs has led to significant increases in desired LME activity [218]. To illustrate the potential of this approach; pathway engineering by Camarero et al., (2012) resulted in an 8000-fold increase in laccase activity in

Pycnoporus cinnabarinus [219]. Ultimately, understanding of such mechanisms could lead to species which have a consistent, high expression of pathways relevant to mycoremediation that are not or less negatively influenced by external factors.

Moreover, recombinant approaches can aid in making EDC-mycoremediation feasible. EDC-mycoremediation associated pathways can be rebuilt into model organisms to increase enzyme production in model fungal (such as *Aspergillus* species) and bacterial species (*E.coli*) to lower enzyme production costs [79].

Furthermore, WRF with shown EDC-remediative capacity can be enhanced by recombinant expression of complementary enzymes and pathways. Several efforts have already been made to recombinantly express foreign LMEs in WRF with mycoremediative capacity. E.g. an inducible gene from *Phlebia tremellosa* has been transformed into *Irpex lacteus*, which led to a 6-fold increase in laccase activity, and increased EDC degrading capacity in transformed strains relative to wild-type [139]. Similar studies for other LMEs also yielded promising results transforming recombinant MnPs to gain higher potential EDC-degrading fungal strains [220].

6. PERSPECTIVES OF WHITE-ROT FUNGAL MEDIATED REMOVAL OF ENDOCRINE DISRUPTIVE COMPOUNDS

Several areas of research have been identified that are likely to influence the development of WRF-based EDC-remediative approaches. Two of these fields –synthetic biology and bio-nanotechnology- and highlighted below as potential key fields to drive mycoremediative systems forward.

6.1 Synthetic biology

As enzyme production is a limiting factor in many biotechnological processes, interest in reducing such limitations is high. One solution to producing enzymes cheaply could be through synthetic biology. To exemplify, Commelas-Aragonès et al., (2007) described a virus-based system that can produce and peroxidase enzymes and control activity upon environmental triggers [221]. Such a system could not only provide possibilities to cheaply produce LMEs but could also provide other advantages to bioremediative systems. The peroxidase enzymes produced by the virus remained encapsidated by the viral particles (VLPs) and were in this manner less bound to denature relative to free enzymes. Nevertheless, the synthesized peroxidases were fully functional, as the VLP allowed substrate and product transfer [221]. Additionally, synthetic biology might contribute to LME-mediator systems. LME-mediator systems have been proposed as an environmentally friendly alternative to chemical oxidative systems [66]. So far, most research has focused on the use of established synthetic mediators. Modern biological tools (synthetic biology) allow for improvement in stability and catalytic activity these mediator compounds by the development of so-called 'designer' mediators to further improve mycoremediative systems [66]. Such designer mediators could be engineered to combine structural properties of potent existing mediator compounds and in this way result in novel compounds with enhanced mediator-function.

6.2 Bio-nanotechnology

Another new, innovative approach is the combination of using nanomaterials and nanotechnology with biotechnology. This novel field is called bio-nanotechnology.

6.2.1 Nanoparticle immobilization

Enhanced stability, activation and reusability of WRF-derived LMEs have earlier been identified as key areas to potentiate mycoremediation [25]. For this reason, the use of nanoparticles to enhance both fungal and enzymatic systems has recently attracted more scientific interest. For enzymatic systems, nanotechnology has been used to create polymer nanofiber filters which allow for high-efficiency enzyme aggregation, whilst improving enzyme stability [205, 222]. WRF LME enzyme activity was found to retain its optimum after a month of use, which is exception relative to other carrier systems [222]. Besides these advantages, reusability seems to be greatly enhanced by using nanoparticles to immobilize enzymes. Demarche et al., (2010) found that the recovery rate of immobilized laccase on nanoparticles neared 100% relative to immobilized laccase on micro (25%) and macroparticles (3%) [223]. The use of nanotechnology for immobilized enzymatic systems has been reviewed by Ansari et al., (2011) and will likely benefit the field in the future [224].

6.2.2 Nanozymes

Another nanotechnology-related field of recent emerging interest is the use of the next generation of artificial enzymes: nanozymes [213]. 'Nanozymes' is the term for nanomaterial that has the same kinetic and physiological functioning as enzymes despite lacking enzymatic active sites. Nanozymes are deemed low cost and highly stable. The first nanozymatic systems have already been developed in the last two years, and more systems which are able to mimic LMEs are bound to be developed. In 2017, Liang et al., managed to mimic laccases by using multicopper coordinated nanomaterial [225]. Such material showed successful conversion of phenolic compounds, and managed to do so with a 2400-fold lower cost, while maintaining similar substrate specificity, higher thermal and pH stability, and retaining activity over longer times relative to the reference laccases in optimal conditions [225]. The authors of the papers hence conclude that prospectively more nanomaterials will be able to replace protein enzymes [225].

7. CONCLUSION

Altogether, EDCs are very diverse, ubiquitous contaminants that have a large-scale negative effect on human and animal health (diseases) and nature (ecological effects). EDCs are found in many products for human consumption, in industrial waste streams, and agriculture. Ultimately, many persistent EDCs end up in our water environments. Current water treatment plants seem to be unadapted to EDCs. Therefore, cost-efficient and environmentally friendly alternatives to combat endocrine disruptive compounds are desired. Bioremediation might provide a remedy for the removal of EDCs. WRFs have a wide arsenal of tools available that are efficient in remediating EDCs that are found in water.

These tools come in the form of naturally occurring pathways and enzymes that can be exploited in systems. Current literature has pointed out that the use of WRFs and their mechanisms are effective in degrading endocrine disrupting compounds in controlled, pilot micro-systems without a heavy focus on optimisation (such as immobilization, system architecture, or bio-engineering). Large-scale pilots that mimic water treatment plants are currently lacking, yet are required as the next step to provide better insight into the feasibility of the technology and the real world application (economics, volumetrics, and safety).

For the future, it is evident that there are still many unaddressed gaps and technological opportunities (highlighted in this review) that will need to be addressed or exploited to further develop the field of EDC-degradation through mycoremediation.

REFERENCES

1. Grześkowiak, T., B. Czarczyńska-Goślińska, and A. Zgoła-Grześkowiak, *Biodegradation of Selected Endocrine Disrupting Compounds*, in *Toxicity and Biodegradation Testing*. 2018, Springer. p. 1-27.
2. Mir-Tutusaus, J.A., et al., *Can white-rot fungi be a real wastewater treatment alternative for organic micropollutants removal? A review.* Water research, 2018.
3. Davis, M., R. Sommer, and J. Menge, *Field guide to mushrooms of western North America*. Vol. 106. 2012: Univ of California Press.
4. Thongprakaisang, S., et al., *Glyphosate induces human breast cancer cells growth via estrogen receptors.* Food and Chemical Toxicology, 2013. **59**: p. 129-136.
5. Kinnunen, A., et al., *Improved efficiency in screening for lignin-modifying peroxidases and laccases of basidiomycetes.* Current Biotechnology, 2017. **6**(2): p. 105-115.
6. Perez-Boada, M., et al., *Versatile peroxidase oxidation of high redox potential aromatic compounds: site-directed mutagenesis, spectroscopic and crystallographic investigation of three long-range electron transfer pathways.* Journal of molecular biology, 2005. **354**(2): p. 385-402.
7. Diamanti-Kandarakis, E., et al., *Endocrine-disrupting chemicals: an Endocrine Society scientific statement.* Endocrine reviews, 2009. **30**(4): p. 293-342.
8. Dallegrave, E., et al., *Pre-and postnatal toxicity of the commercial glyphosate formulation in Wistar rats.* Archives of toxicology, 2007. **81**(9): p. 665-673.
9. Kim, Y., et al., *Removal of estrogenic activity from endocrine-disrupting chemicals by purified laccase of Phlebia tremellosa.* FEMS microbiology letters, 2008. **284**(2): p. 172-175.
10. Esplugas, S., et al., *Ozonation and advanced oxidation technologies to remove endocrine disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs) in water effluents.* Journal of hazardous materials, 2007. **149**(3): p. 631-642.
11. Vares, T., M. Kalsi, and A. Hatakka, *Lignin Peroxidases, Manganese Peroxidases, and Other Ligninolytic Enzymes Produced by Phlebia radiata during Solid-State Fermentation of Wheat Straw.* Applied and Environmental Microbiology, 1995. **61**(10): p. 3515-3520.
12. J. R. Rice, P.D., S. Ramaiahgari, S. Ferguson, S. L. Smith-Roe, and M. DeVito. , *Effects Of Glyphosate And Its Formulations On Markers Of Oxidative Stress And Cell Viability In HepaRG And HaCaT Cell Lines.* 2018.
13. Di Prisco, G., et al., *Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees.* Proceedings of the National Academy of Sciences, 2013. **110**(46): p. 18466-18471.
14. Kortenkamp, A., *Ten years of mixing cocktails: a review of combination effects of endocrine-disrupting chemicals.* Environmental health perspectives, 2007. **115**(Suppl 1): p. 98.
15. Cooper, C.E. and G.C. Brown, *The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: chemical mechanism and physiological significance.* Journal of bioenergetics and biomembranes, 2008. **40**(5): p. 533.
16. Wogan, G. and P. Newberne, *Dose-response characteristics of aflatoxin B1 carcinogenesis in the rat.* Cancer research, 1967. **27**(1): p. 2370-2376.
17. Marieb, E.N. and K. Hoehn, *Human anatomy & physiology*. 2007: Pearson Education.
18. Iavicoli, I., L. Fontana, and A. Bergamaschi, *The effects of metals as endocrine disruptors.* Journal of Toxicology and Environmental Health, Part B, 2009. **12**(3): p. 206-223.
19. De Toni, L., et al., *Phthalates and heavy metals as endocrine disruptors in food: A study on pre-packed coffee products.* Toxicology reports, 2017. **4**: p. 234-239.
20. Kolpin, D.W., et al., *Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999− 2000: A national reconnaissance.* Environmental science & technology, 2002. **36**(6): p. 1202-1211.
21. Heberer, T., *Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data.* Toxicology letters, 2002. **131**(1-2): p. 5-17.
22. Muncke, J., *Exposure to endocrine disrupting compounds via the food chain: Is packaging a relevant source?* Science of the total environment, 2009. **407**(16): p. 4549-4559.
23. Hatakka, A., *Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation.* FEMS microbiology reviews, 1994. **13**(2-3): p. 125-135.
24. Pointing, S., *Feasibility of bioremediation by white-rot fungi.* Applied microbiology and biotechnology, 2001. **57**(1-2): p. 20-33.
25. Asgher, M., et al., *Recent developments in biodegradation of industrial pollutants by white rot fungi and their enzyme system.* Biodegradation, 2008. **19**(6): p. 771.
26. Barr, D.P. and S.D. Aust, *Mechanisms white rot fungi use to degrade pollutants.* Environmental Science & Technology, 1994. **28**(2): p. 78A-87A.
27. Wesenberg, D., I. Kyriakides, and S.N. Agathos, *White-rot fungi and their enzymes for the treatment of industrial dye effluents.* Biotechnology advances, 2003. **22**(1-2): p. 161-187.
28. Robinson, T., et al., *Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative.* Bioresource technology, 2001. **77**(3): p. 247-255.
29. Robinson, T. and P.S. Nigam, *Remediation of textile dye waste water using a white-rot fungus Bjerkandera adusta through solid-state fermentation (SSF).* Applied biochemistry and biotechnology, 2008. **151**(2-3): p. 618.
30. Cabana, H., J. Jones, and S.N. Agathos, *Elimination of endocrine disrupting chemicals using white rot fungi and their lignin modifying enzymes: a review.* Engineering in Life Sciences, 2007. **7**(5): p. 429-456.

31. Soares, A., et al., *The ability of white-rot fungi to degrade the endocrine-disrupting compound nonylphenol.* Applied microbiology and biotechnology, 2005. **66**(6): p. 719-725.
32. Cajthaml, T., et al., *Biodegradation of endocrine-disrupting compounds and suppression of estrogenic activity by ligninolytic fungi.* Chemosphere, 2009. **75**(6): p. 745-750.
33. Caliman, F.A. and M. Gavrilescu, *Pharmaceuticals, personal care products and endocrine disrupting agents in the environment–a review.* CLEAN–Soil, Air, Water, 2009. **37**(4‐5): p. 277-303.
34. Gore, A., et al., *Introduction to endocrine disrupting chemicals (EDCs)—a guide for public interest organizations and policy makers.* Endocrine Society reports and white papers, 2014. **1**: p. 76.
35. Dodson, R.E., et al., *Endocrine disruptors and asthma-associated chemicals in consumer products.* Environmental health perspectives, 2012. **120**(7): p. 935.
36. Benotti, M.J., et al., *Pharmaceuticals and endocrine disrupting compounds in US drinking water.* Environmental science & technology, 2008. **43**(3): p. 597-603.
37. Lv, M., et al., *Occurrence and fate of triclosan and triclocarban in a subtropical river and its estuary.* Marine pollution bulletin, 2014. **88**(1-2): p. 383-388.
38. Kassotis, C.D., et al., *Endocrine disrupting activities of surface water associated with a West Virginia oil and gas industry wastewater disposal site.* Science of the Total Environment, 2016. **557**: p. 901-910.
39. White, R., et al., *Environmentally persistent alkylphenolic compounds are estrogenic.* Endocrinology, 1994. **135**(1): p. 175-182.
40. Hayes, T.B., et al., *Pesticide mixtures, endocrine disruption, and amphibian declines: are we underestimating the impact?* Environmental health perspectives, 2006. **114**(Suppl 1): p. 40.
41. Cherniaev, A.P., A.S. Kondakova, and E.N. Zyk, *Contents of 4-nonylphenol in surface sea water of Amur Bay (Japan/East Sea).* Achievements in the Life Sciences, 2016. **10**(1): p. 65-71.
42. Dan, L., et al., *Distribution and bioaccumulation of endocrine disrupting chemicals in water, sediment and fishes in a shallow Chinese freshwater lake: Implications for ecological and human health risks.* Ecotoxicology and Environmental Safety, 2017. **140**: p. 222-229.
43. Battaglin, W.A. and A. Kolok, *Featured collection introduction: Contaminants of emerging concern II.* JAWRA Journal of the American Water Resources Association, 2014. **50**(2): p. 261-265.
44. Kabir, E.R., M.S. Rahman, and I. Rahman, *A review on endocrine disruptors and their possible impacts on human health.* Environmental Toxicology and Pharmacology, 2015. **40**(1): p. 241-258.
45. Skinner, M.K., M. Manikkam, and C. Guerrero-Bosagna, *Epigenetic transgenerational actions of endocrine disruptors.* Reproductive toxicology, 2011. **31**(3): p. 337-343.
46. Trasande, L., et al., *Estimating burden and disease costs of exposure to endocrine-disrupting chemicals in the European Union.* The Journal of Clinical Endocrinology & Metabolism, 2015. **100**(4): p. 1245-1255.
47. Cooke, P.S., L. Simon, and N.D. Denslow, *Chapter 37 - Endocrine Disruptors*, in *Haschek and Rousseaux's Handbook of Toxicologic Pathology (Third Edition)*, W.M. Haschek, C.G. Rousseaux, and M.A. Wallig, Editors. 2013, Academic Press: Boston. p. 1123-1154.
48. Newbold, R., *Cellular and molecular effects of developmental exposure to diethylstilbestrol: implications for other environmental estrogens.* Environmental health perspectives, 1995. **103**(Suppl 7): p. 83.
49. Kidd, K.A., et al., *Human and wildlife exposures to EDCs.* State of the Science of Endocrine Disrupting Chemicals-2012, 2012: p. 1-261.
50. Rudel, R.A. and L.J. Perovich, *Endocrine disrupting chemicals in indoor and outdoor air.* Atmospheric Environment, 2009. **43**(1): p. 170-181.
51. Rudel, R.A., et al., *Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust.* Environmental science & technology, 2003. **37**(20): p. 4543-4553.
52. Pojana, G., et al., *Natural and synthetic endocrine disrupting compounds (EDCs) in water, sediment and biota of a coastal lagoon.* Environment International, 2007. **33**(7): p. 929-936.
53. Colucci, M.S., H. Bork, and E. Topp, *Persistence of estrogenic hormones in agricultural soils.* Journal of Environmental Quality, 2001. **30**(6): p. 2070-2076.
54. Ruhí, A., et al., *Bioaccumulation and trophic magnification of pharmaceuticals and endocrine disruptors in a Mediterranean river food web.* Science of the Total Environment, 2016. **540**: p. 250-259.
55. Thurston, C.F., *The structure and function of fungal laccases.* Microbiology, 1994. **140**(1): p. 19-26.
56. Rivera-Hoyos, C.M., et al., *Fungal laccases.* Fungal Biology Reviews, 2013. **27**(3-4): p. 67-82.
57. Riva, S., *Laccases: blue enzymes for green chemistry.* TRENDS in Biotechnology, 2006. **24**(5): p. 219-226.
58. Hamid, M., *Potential applications of peroxidases.* Food chemistry, 2009. **115**(4): p. 1177-1186.
59. Mot, A. and R. Silaghi-Dumitrescu, *Laccases: complex architectures for one-electron oxidations.* Biochemistry (Moscow), 2012. **77**(12): p. 1395-1407.
60. Couto, S.R. and J.L.T. Herrera, *Industrial and biotechnological applications of laccases: a review.* Biotechnology advances, 2006. **24**(5): p. 500-513.
61. Piontek, K., M. Antorini, and T. Choinowski, *Crystal structure of a laccase from the fungusTrametes versicolor at 1.90-Å resolution containing a full complement of coppers.* Journal of Biological Chemistry, 2002. **277**(40): p. 37663-37669.
62. Baldrian, P., *Fungal laccases–occurrence and properties.* FEMS microbiology reviews, 2006. **30**(2): p. 215-242.
63. Eggert, C., U. Temp, and K.-E.L. Eriksson, *Laccase is essential for lignin degradation by the white‐rot fungus Pycnoporus cinnabarinus.* Febs Letters, 1997. **407**(1): p. 89-92.
64. Mayer, A.M. and R.C. Staples, *Laccase: new functions for an old enzyme.* Phytochemistry, 2002. **60**(6): p. 551-565.

65. Eggert, C., U. Temp, and K.-E. Eriksson, *The ligninolytic system of the white rot fungus Pycnoporus cinnabarinus: purification and characterization of the laccase.* Applied and Environmental Microbiology, 1996. **62**(4): p. 1151-1158.
66. Wells, A., M. Teria, and T. Eve, *Green oxidations with laccase–mediator systems*. 2006, Portland Press Limited.
67. Madhavi, V. and S. Lele, *Laccase: properties and applications.* BioResources, 2009. **4**(4): p. 1694-1717.
68. Barrios-Estrada, C., et al., *Emergent contaminants: Endocrine disruptors and their laccase-assisted degradation–A review.* Science of the Total Environment, 2018. **612**: p. 1516-1531.
69. Cañas, A.I. and S. Camarero, *Laccases and their natural mediators: biotechnological tools for sustainable eco-friendly processes.* Biotechnology advances, 2010. **28**(6): p. 694-705.
70. Li, H., et al., *Determinants of the relative reduction potentials of type-1 copper sites in proteins.* Journal of the American Chemical Society, 2004. **126**(25): p. 8010-8019.
71. Cabana, H., et al., *Immobilization of laccase from the white rot fungus Coriolopsis polyzona and use of the immobilized biocatalyst for the continuous elimination of endocrine disrupting chemicals.* Bioresource technology, 2009. **100**(14): p. 3447-3458.
72. Keum, Y.S. and Q.X. Li, *Fungal laccase-catalyzed degradation of hydroxy polychlorinated biphenyls.* Chemosphere, 2004. **56**(1): p. 23-30.
73. Nguyen, L.N., et al., *Removal of pharmaceuticals, steroid hormones, phytoestrogens, UV-filters, industrial chemicals and pesticides by Trametes versicolor: role of biosorption and biodegradation.* International Biodeterioration & Biodegradation, 2014. **88**: p. 169-175.
74. Houtman, C.J., et al., *Fungal lignin peroxidase does not produce the veratryl alcohol cation radical as a diffusible ligninolytic oxidant.* Journal of Biological Chemistry, 2018: p. jbc. RA117. 001153.
75. Hammel, K.E. and D. Cullen, *Role of fungal peroxidases in biological ligninolysis.* Current opinion in plant biology, 2008. **11**(3): p. 349-355.
76. Wong, D.W., *Structure and action mechanism of ligninolytic enzymes.* Applied biochemistry and biotechnology, 2009. **157**(2): p. 174-209.
77. Martınez, A.T., *Molecular biology and structure-function of lignin-degrading heme peroxidases.* Enzyme and Microbial Technology, 2002. **30**(4): p. 425-444.
78. Wang, J., et al., *Effective removal of endocrine-disrupting compounds by lignin peroxidase from the white-rot fungus Phanerochaete sordida YK-624.* Current microbiology, 2012. **64**(3): p. 300-303.
79. Hofrichter, M., *lignin conversion by manganese peroxidase (MnP).* Enzyme and Microbial technology, 2002. **30**(4): p. 454-466.
80. Mohammadi, A. and B. Nasernejad, *Enzymatic degradation of anthracene by the white rot fungus Phanerochaete chrysosporium immobilized on sugarcane bagasse.* Journal of Hazardous Materials, 2009. **161**(1): p. 534-537.
81. Hirano, T., et al., *Degradation of bisphenol A by the lignin-degrading enzyme, manganese peroxidase, produced by the white-rot basidiomycete, Pleurotus ostreatus.* Bioscience, biotechnology, and biochemistry, 2000. **64**(9): p. 1958-1962.
82. Dashtban, M., et al., *Fungal biodegradation and enzymatic modification of lignin.* International journal of biochemistry and molecular biology, 2010. **1**(1): p. 36.
83. Taboada-Puig, R., et al., *A new strain of Bjerkandera sp. production, purification and characterization of versatile peroxidase.* World Journal of Microbiology and Biotechnology, 2011. **27**(1): p. 115-122.
84. Eibes, G., et al., *Oxidation of pharmaceutically active compounds by a ligninolytic fungal peroxidase.* Biodegradation, 2011. **22**(3): p. 539-550.
85. Taboada-Puig, R., et al., *Combined cross-linked enzyme aggregates from versatile peroxidase and glucose oxidase: production, partial characterization and application for the elimination of endocrine disruptors.* Bioresource technology, 2011. **102**(11): p. 6593-6599.
86. Wu, Y., et al., *Potential role of polycyclic aromatic hydrocarbons (PAHs) oxidation by fungal laccase in the remediation of an aged contaminated soil.* Soil Biology and Biochemistry, 2008. **40**(3): p. 789-796.
87. Wen, X., Y. Jia, and J. Li, *Degradation of tetracycline and oxytetracycline by crude lignin peroxidase prepared from Phanerochaete chrysosporium–a white rot fungus.* Chemosphere, 2009. **75**(8): p. 1003-1007.
88. Teunissen, P.J. and J.A. Field, *2 - Chloro - 1, 4 - dimethoxybenzene as a mediator of lignin peroxidase catalyzed oxidations.* FEBS letters, 1998. **439**(3): p. 219-223.
89. Michizoe, J., et al., *Activation of manganese peroxidase in an organic medium using a mediator.* Biochemical engineering journal, 2004. **19**(1): p. 43-46.
90. Taboada-Puig, R., et al., *Fostering the action of versatile peroxidase as a highly efficient biocatalyst for the removal of endocrine disrupting compounds.* New biotechnology, 2016. **33**(1): p. 187-195.
91. Leonowicz, A., et al., *Biodegradation of lignin by white rot fungi.* Fungal genetics and biology, 1999. **27**(2-3): p. 175-185.
92. Marzullo, L., et al., *Veratryl alcohol oxidase from Pleurotus ostreatus participates in lignin biodegradation and prevents polymerization of laccase-oxidized substrates.* Journal of Biological Chemistry, 1995. **270**(8): p. 3823-3827.
93. Marco-Urrea, E., et al., *Ability of white-rot fungi to remove selected pharmaceuticals and identification of degradation products of ibuprofen by Trametes versicolor.* Chemosphere, 2009. **74**(6): p. 765-772.
94. Westereng, B., et al., *Enzymatic cellulose oxidation is linked to lignin by long-range electron transfer.* Scientific reports, 2015. **5**: p. 18561.
95. Fabbrini, M., C. Galli, and P. Gentili, *Comparing the catalytic efficiency of some mediators of laccase.* Journal of Molecular Catalysis B: Enzymatic, 2002. **16**(5-6): p. 231-240.

96. Husain, M. and Q. Husain, *Applications of redox mediators in the treatment of organic pollutants by using oxidoreductive enzymes: a review.* Critical Reviews in Environmental Science and Technology, 2007. **38**(1): p. 1-42.
97. Camarero, S., et al., *Lignin-derived compounds as efficient laccase mediators for decolorization of different types of recalcitrant dyes.* Applied and environmental microbiology, 2005. **71**(4): p. 1775-1784.
98. Ibarra, D., et al., *Exploring the enzymatic parameters for optimal delignification of eucalypt pulp by laccase-mediator.* Enzyme and Microbial Technology, 2006. **39**(6): p. 1319-1327.
99. Nishizawa, Y., K. Nakabayashi, and E. Shinagawa, *Purification and characterization of laccase from white rot fungus Trametes sanguinea M85-2.* Journal of Fermentation and Bioengineering, 1995. **80**(1): p. 91-93.
100. Rodakiewicz-Nowak, J., A. Jarosz-Wilkołazka, and J. Luterek, *Catalytic activity of versatile peroxidase from Bjerkandera fumosa in aqueous solutions of water–miscible organic solvents.* Applied Catalysis A: General, 2006. **308**: p. 56-61.
101. da Silva Coelho-Moreira, J., et al., *Involvement of lignin-modifying enzymes in the degradation of herbicides*, in *Herbicides-Advances in Research*. 2013, InTech.
102. Pelaez, F., M.J. Martinez, and A. Martinez, *Screening of 68 species of basidiomycetes for enzymes involved in lignin degradation.* Mycological research, 1995. **99**(1): p. 37-42.
103. Hatakka, A., *Lignin-modifying enzymes fungi: production and role from selected white-rot in lignin degradation.* FEMS Microbiol. Rev, 1994. **13**: p. 125-135.
104. Morozova, O., et al., *"Blue" laccases.* Biochemistry (Moscow), 2007. **72**(10): p. 1136-1150.
105. Daâssi, D., et al., *Degradation of bisphenol A by different fungal laccases and identification of its degradation products.* International Biodeterioration & Biodegradation, 2016. **110**: p. 181-188.
106. Yang, J., et al., *Laccase production and differential transcription of laccase genes in Cerrena sp. in response to metal ions, aromatic compounds, and nutrients.* Frontiers in microbiology, 2016. **6**: p. 1558.
107. Yang, J., et al., *Laccase gene family in Cerrena sp. HYB07: sequences, heterologous expression and transcriptional analysis.* Molecules, 2016. **21**(8): p. 1017.
108. Songulashvili, G., et al., *Immobilized laccase of Cerrena unicolor for elimination of endocrine disruptor micropollutants.* Fungal biology, 2012. **116**(8): p. 883-889.
109. Elisashvili, V., et al., *Physiological Peculiarities of Lignin-Modifying Enzyme Production by the White-Rot Basidiomycete Coriolopsis gallica Strain BCC 142.* Microorganisms, 2017. **5**(4): p. 73.
110. Kachlishvili, E., E. Metreveli, and V. Elisashvili, *Modulation of Cerrena unicolor laccase and manganese peroxidase production.* SpringerPlus, 2014. **3**(1): p. 463.
111. Winquist, E., et al., *Production of lignin modifying enzymes on industrial waste material by solid-state cultivation of fungi.* Biochemical Engineering Journal, 2008. **42**(2): p. 128-132.
112. Cabana, H., et al., *Elimination of endocrine disrupting chemicals nonylphenol and bisphenol A and personal care product ingredient triclosan using enzyme preparation from the white rot fungus Coriolopsis polyzona.* Chemosphere, 2007. **67**(4): p. 770-778.
113. Cabana, H., J.P. Jones, and S.N. Agathos, *Preparation and characterization of cross-linked laccase aggregates and their application to the elimination of endocrine disrupting chemicals.* Journal of Biotechnology, 2007. **132**(1): p. 23-31.
114. Jaouani, A., M.G. Tabka, and M.J. Penninckx, *Lignin modifying enzymes of Coriolopsis polyzona and their role in olive oil mill wastewaters decolourisation.* Chemosphere, 2006. **62**(9): p. 1421-1430.
115. Šušla, M., et al., *Implication of Dichomitus squalens manganese-dependent peroxidase in dye decolorization and cooperation of the enzyme with laccase.* Folia microbiologica, 2008. **53**(6): p. 479-485.
116. Périé, F.H., et al., *Purification and Characterization of Laccases from the White-Rot BasidiomyceteDichomitus squalens.* Archives of biochemistry and biophysics, 1998. **353**(2): p. 349-355.
117. Agrawal, N., P. Verma, and S.K. Shahi, *Degradation of polycyclic aromatic hydrocarbons (phenanthrene and pyrene) by the ligninolytic fungi Ganoderma lucidum isolated from the hardwood stump.* Bioresources and Bioprocessing, 2018. **5**(1): p. 11.
118. Kaur, H., S. Kapoor, and G. Kaur, *Application of ligninolytic potentials of a white-rot fungus Ganoderma lucidum for degradation of lindane.* Environmental monitoring and assessment, 2016. **188**(10): p. 588.
119. Shin, E., H.T. Choi, and H. Song, *Biodegradation of endocrine-disrupting bisphenol A by white rot fungus Irpex lacteus.* Journal of microbiology and biotechnology, 2007. **17**(7): p. 1147.
120. Moon, D.-S. and H.-G. Song, *Degradation of alkylphenols by white rot fungus Irpex lacteus and its manganese peroxidase.* Applied biochemistry and biotechnology, 2012. **168**(3): p. 542-549.
121. Novotný, Č., et al., *Irpex lacteus, a white-rot fungus with biotechnological potential.* Folia Microbiologica, 2009. **54**(5): p. 375-390.
122. Chen, W., et al., *Cloning and expression of a new manganese peroxidase from Irpex lacteus F17 and its application in decolorization of reactive black 5.* Process Biochemistry, 2015. **50**(11): p. 1748-1759.
123. Kordon, K., A. Mikolasch, and F. Schauer, *Oxidative dehalogenation of chlorinated hydroxybiphenyls by laccases of white-rot fungi.* International Biodeterioration & Biodegradation, 2010. **64**(3): p. 203-209.
124. Syed, K., et al., *Genome-wide identification, annotation and characterization of novel thermostable cytochrome P450 monooxygenases from the thermophilic biomass-degrading fungi Thielavia terrestris and Myceliophthora thermophila.* Genes & Genomics, 2014. **36**(3): p. 321-333.
125. Singh, B., *Myceliophthora thermophila syn. Sporotrichum thermophile: a thermophilic mould of biotechnological potential.* Critical reviews in biotechnology, 2016. **36**(1): p. 59-69.
126. D'Annibale, A., et al., *Panus tigrinus efficiently removes phenols, color and organic load from olive-mill wastewater.* Research in Microbiology, 2004. **155**(7): p. 596-603.
127. Quaratino, D., et al., *Production, purification and partial characterisation of a novel laccase from the white-rot fungus Panus tigrinus CBS 577.79.* Antonie van Leeuwenhoek, 2007. **91**(1): p. 57-69.

128. Jhadav, A., et al., *Optimization of production and partial purification of laccase by Phanerochaete chrysosporium using submerged fermenation.* International Journal of Microbiology Research, 2009. **1**(2): p. 9.
129. Martinez, D., et al., *Genome sequence of the lignocellulose degrading fungus Phanerochaete chrysosporium strain RP78.* Nature biotechnology, 2004. **22**(6): p. 695.
130. Bonnarme, P. and T.W. Jeffries, *Mn (II) regulation of lignin peroxidases and manganese-dependent peroxidases from lignin-degrading white rot fungi.* Applied and Environmental Microbiology, 1990. **56**(1): p. 210-217.
131. Ainsworth, A. and A. Rayner, *Ontogenetic stages from coenocyte to basidiome and their relation to phenoloxidase activity and colonization processes in Phanerochaete magnoliae.* Mycological Research, 1991. **95**(12): p. 1414-1422.
132. Tamagawa, Y., et al., *Removal of estrogenic activity of natural steroidal hormone estrone by ligninolytic enzymes from white rot fungi.* Chemosphere, 2006. **65**(1): p. 97-101.
133. Tamagawa, Y., et al., *Removal of estrogenic activity of endocrine-disrupting genistein by ligninolytic enzymes from white rot fungi.* FEMS microbiology letters, 2005. **244**(1): p. 93-98.
134. Sugiura, M., H. Hirai, and T. Nishida, *Purification and characterization of a novel lignin peroxidase from white-rot fungus Phanerochaete sordida YK-624.* FEMS microbiology letters, 2003. **224**(2): p. 285-290.
135. Hirai, H., et al., *Characteristics of novel lignin peroxidases produced by white-rot fungus Phanerochaete sordida YK-624.* FEMS microbiology letters, 2005. **246**(1): p. 19-24.
136. Lee, H., et al., *Biotechnological procedures to select white rot fungi for the degradation of PAHs.* Journal of Microbiological Methods, 2014. **97**: p. 56-62.
137. Lee, A.H., H. Lee, and J.-J. Kim, *Simultaneous Degradation of Polycyclic Aromatic Hydrocarbons by Attractive Ligninolytic Enzymes from Phlebia brevispora KUC9045.* 환경생물, 2016. **34**(3): p. 201-207.
138. Kamei, I., et al., *Fungal bioconversion of toxic polychlorinated biphenyls by white-rot fungus, Phlebia brevispora.* Applied microbiology and biotechnology, 2006. **73**(4): p. 932-940.
139. Kum, H., M.K. Kim, and H.T. Choi, *Degradation of endocrine disrupting chemicals by genetic transformants in Irpex lacteus with an inducible laccase gene of Phlebia tremellosa.* Biodegradation, 2009. **20**(5): p. 673.
140. Robinson, T., B. Chandran, and P. Nigam, *Studies on the production of enzymes by white-rot fungi for the decolourisation of textile dyes.* Enzyme and Microbial technology, 2001. **29**(8-9): p. 575-579.
141. Hadibarata, T. and R.A. Kristanti, *Potential of a white-rot fungus Pleurotus eryngii F032 for degradation and transformation of fluorene.* Fungal biology, 2014. **118**(2): p. 222-227.
142. Pérez-Boada, M., et al., *Expression of Pleurotus eryngii versatile peroxidase in Escherichia coli and optimisation of in vitro folding.* Enzyme and Microbial Technology, 2002. **30**(4): p. 518-524.
143. Martínez, M.J., et al., *Purification and catalytic properties of two manganese peroxidase isoenzymes from Pleurotus eryngii.* European Journal of Biochemistry, 1996. **237**(2): p. 424-432.
144. Munoz, C., et al., *Laccase isoenzymes of Pleurotus eryngii: characterization, catalytic properties, and participation in activation of molecular oxygen and Mn2+ oxidation.* Applied and Environmental Microbiology, 1997. **63**(6): p. 2166-2174.
145. Ueda, M., et al., *A protein from Pleurotus eryngii var. tuoliensis CJ Mou with strong removal activity against the natural steroid hormone, estriol: purification, characterization, and identification as a laccase.* Enzyme and microbial technology, 2012. **51**(6-7): p. 402-407.
146. Purnomo, A.S., et al., *Application of mushroom waste medium from Pleurotus ostreatus for bioremediation of DDT-contaminated soil.* International Biodeterioration & Biodegradation, 2010. **64**(5): p. 397-402.
147. Ahuactzin-Pérez, M., et al., *A novel biodegradation pathway of the endocrine-disruptor di (2-ethyl hexyl) phthalate by Pleurotus ostreatus based on quantum chemical investigation.* Ecotoxicology and environmental safety, 2018. **147**: p. 494-499.
148. de Freitas, E.N., et al., *Removal of bisphenol A by laccases from Pleurotus ostreatus and Pleurotus pulmonarius and evaluation of ecotoxicity of degradation products.* Chemical Engineering Journal, 2017. **330**: p. 1361-1369.
149. Rodrıguez, E., et al., *Degradation of phenolic and non-phenolic aromatic pollutants by four Pleurotus species: the role of laccase and versatile peroxidase.* Soil Biology and Biochemistry, 2004. **36**(6): p. 909-916.
150. Hildén, K., et al., *Heterologous expression and structural characterization of two low pH laccases from a biopulping white-rot fungus Physisporinus rivulosus.* Applied microbiology and biotechnology, 2013. **97**(4): p. 1589-1599.
151. Le, T.N.U., *Degradation of bisphenol A (BPA) by fungal laccase-mediator systems.* 2017.
152. Hakala, T.K., et al., *Differential regulation of manganese peroxidases and characterization of two variable MnP encoding genes in the white-rot fungus Physisporinus rivulosus.* Applied microbiology and biotechnology, 2006. **73**(4): p. 839-849.
153. Hakala, T., *Characterization of lignin-modifying enzymes of the selective white-rot fungus Physisporinus rivulosus.* 2007.
154. Hildén, K., et al., *Novel thermotolerant laccases produced by the white-rot fungus Physisporinus rivulosus.* Applied microbiology and biotechnology, 2007. **77**(2): p. 301-309.
155. Lloret, L., et al., *Immobilisation of laccase on Eupergit supports and its application for the removal of endocrine disrupting chemicals in a packed-bed reactor.* Biodegradation, 2012. **23**(3): p. 373-386.
156. Ramírez-Cavazos, L.I., et al., *Purification and characterization of two thermostable laccases from Pycnoporus sanguineus and potential role in degradation of endocrine disrupting chemicals.* Journal of Molecular Catalysis B: Enzymatic, 2014. **108**: p. 32-42.

157. Wang, Z.-X., et al., *Purification and characterization of two thermostable laccases with high cold adapted characteristics from Pycnoporus sp. SYBC-L1.* Process Biochemistry, 2010. **45**(10): p. 1720-1729.
158. Hidayat, A. and D.H.Y. Yanto, *Biodegradation and metabolic pathway of phenanthrene by a new tropical fungus, Trametes hirsuta D7.* Journal of Environmental Chemical Engineering, 2018. **6**(2): p. 2454-2460.
159. Ike, M., et al., *FUNGAL BIOREACTOR WITH ULTRAMEMBRANE SEPARATION FOR DEGRADATION OF COLORED-AND ENDOCRINE DISRUPTING-SUBSTANCES.* Annual Report of FY 2004, The Core University Program between Japan Society for the Promotion of Science (JSPS) and Vietnamese Academy of Science and Technology (VAST), 2005: p. 155-158.
160. Chairin, T., et al., *Biodegradation of bisphenol A and decolorization of synthetic dyes by laccase from white-rot fungus, Trametes polyzona.* Applied biochemistry and biotechnology, 2013. **169**(2): p. 539-545.
161. Teerapatsakul, C., et al., *Biodegradation of polycyclic aromatic hydrocarbons by a thermotolerant white rot fungus Trametes polyzona RYNF13.* The Journal of general and applied microbiology, 2016. **62**(6): p. 303-312.
162. Spina, F., et al., *Removal of micropollutants by fungal laccases in model solution and municipal wastewater: evaluation of estrogenic activity and ecotoxicity.* Journal of Cleaner Production, 2015. **100**: p. 185-194.
163. Levin, L., A. Viale, and A. Forchiassin, *Degradation of organic pollutants by the white rot basidiomycete Trametes trogii.* International Biodeterioration & Biodegradation, 2003. **52**(1): p. 1-5.
164. Levin, L. and F. Forchiassin, *Ligninolytic enzymes of the white rot basidiomycete Trametes trogii.* Acta Biotechnologica, 2001. **21**(2): p. 179-186.
165. Kim, Y., et al., *Enhanced expression of laccase during the degradation of endocrine disrupting chemicals in Trametes versicolor.* The Journal of Microbiology, 2008. **46**(4): p. 402.
166. Asgher, M., H.M.N. Iqbal, and M. Irshad, *Characterization of purified and xerogel immobilized novel lignin peroxidase produced from Trametes versicolor IBL-04 using solid state medium of corncobs.* BMC biotechnology, 2012. **12**(1): p. 46.
167. Pezzella, C., et al., *Exploitation of Trametes versicolor for bioremediation of endocrine disrupting chemicals in bioreactors.* PloS one, 2017. **12**(6): p. e0178758.
168. Fukuda, T., et al., *Degradation of bisphenol A by purified laccase from Trametes villosa.* Biochemical and biophysical research communications, 2001. **284**(3): p. 704-706.
169. Silva, M.L.C., et al., *Production of manganese peroxidase by Trametes villosa on unexpensive substrate and its application in the removal of lignin from agricultural wastes.* Advances in Bioscience and Biotechnology, 2014. **5**(14): p. 1067.
170. Yaver, D.S. and E.J. Golightly, *Cloning and characterization of three laccase genes from the white-rot basidiomycete Trametes villosa: genomic organization of the laccase gene family.* Gene, 1996. **181**(1): p. 95-102.
171. Bolong, N., et al., *A review of the effects of emerging contaminants in wastewater and options for their removal.* Desalination, 2009. **239**(1-3): p. 229-246.
172. Cesaro, A. and V. Belgiorno, *Removal of endocrine disruptors from urban wastewater by advanced oxidation processes (AOPs): a review.* The Open Biotechnology Journal, 2016. **10**(1).
173. Pandey, J., A. Chauhan, and R.K. Jain, *Integrative approaches for assessing the ecological sustainability of in situ bioremediation.* FEMS Microbiology Reviews, 2009. **33**(2): p. 324-375.
174. Hancock, N.T., et al., *Comprehensive bench-and pilot-scale investigation of trace organic compounds rejection by forward osmosis.* Environmental science & technology, 2011. **45**(19): p. 8483-8490.
175. Verma, P. and D. Madamwar, *Production of ligninolytic enzymes for dye decolorization by cocultivation of white-rot fungi Pleurotus ostreatus and Phanerochaete chrysosporium under solid-state fermentation.* Applied biochemistry and biotechnology, 2002. **102**(1-6): p. 109-118.
176. Yang, S., et al., *Understanding the factors controlling the removal of trace organic contaminants by white-rot fungi and their lignin modifying enzymes: a critical review.* Bioresource technology, 2013. **141**: p. 97-108.
177. Choi, K.J., et al., *Effects of activated carbon types and service life on removal of endocrine disrupting chemicals: amitrol, nonylphenol, and bisphenol-A.* Chemosphere, 2005. **58**(11): p. 1535-1545.
178. Freihardt, J., M. Jekel, and A.S. Ruhl, *Comparing test methods for granular activated carbon for organic micropollutant elimination.* Journal of environmental chemical engineering, 2017. **5**(3): p. 2542-2551.
179. Snyder, S.A., et al., *Ozone oxidation of endocrine disruptors and pharmaceuticals in surface water and wastewater.* Ozone: Science and Engineering, 2006. **28**(6): p. 445-460.
180. Yoon, Y., et al., *Removal of endocrine disrupting compounds and pharmaceuticals by nanofiltration and ultrafiltration membranes.* Desalination, 2007. **202**(1-3): p. 16-23.
181. Braeken, L. and B. Van der Bruggen, *Feasibility of nanofiltration for the removal of endocrine disrupting compounds.* Desalination, 2009. **240**(1-3): p. 127-131.
182. Jamaly, S., et al., *A short review on reverse osmosis pretreatment technologies.* Desalination, 2014. **354**: p. 30-38.
183. Azubuike, C.C., C.B. Chikere, and G.C. Okpokwasili, *Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects.* World Journal of Microbiology and Biotechnology, 2016. **32**(11): p. 180.
184. Nakada, N., et al., *Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment.* Water research, 2006. **40**(17): p. 3297-3303.
185. Janusz, G., et al., *Fungal laccase, manganese peroxidase and lignin peroxidase: gene expression and regulation.* Enzyme and microbial technology, 2013. **52**(1): p. 1-12.
186. Keyser, P., T. Kirk, and J. Zeikus, *Ligninolytic enzyme system of Phanaerochaete chrysosporium: synthesized in the absence of lignin in response to nitrogen starvation.* Journal of bacteriology, 1978. **135**(3): p. 790-797.

187. Komori, K., et al., *Analysis and occurrence of estrogen in wastewater in Japan.* Water Science and Technology, 2004. **50**(5): p. 93-100.
188. Asif, M.B., et al., *Degradation of pharmaceuticals and personal care products by white-rot fungi—A critical review.* Current Pollution Reports, 2017. **3**(2): p. 88-103.
189. Zhang, Y. and S.-U. Geißen, *Elimination of carbamazepine in a non-sterile fungal bioreactor.* Bioresource technology, 2012. **112**: p. 221-227.
190. Libra, J.A., M. Borchert, and S. Banit, *Competition strategies for the decolorization of a textile‐reactive dye with the white‐rot fungi Trametes versicolor under non‐sterile conditions.* Biotechnology and Bioengineering, 2003. **82**(6): p. 736-744.
191. Křesinová, Z., et al., *Biodegradation of endocrine disruptors in urban wastewater using Pleurotus ostreatus bioreactor.* New biotechnology, 2018. **43**: p. 53-61.
192. Shreve, M.J., et al., *The white-rot fungus Trametes versicolor reduces the estrogenic activity of a mixture of emerging contaminants in wastewater treatment plant effluent.* International Biodeterioration & Biodegradation, 2016. **109**: p. 132-140.
193. Mir-Tutusaus, J., M. Sarrà, and G. Caminal, *Continuous treatment of non-sterile hospital wastewater by Trametes versicolor: How to increase fungal viability by means of operational strategies and pretreatments.* Journal of hazardous materials, 2016. **318**: p. 561-570.
194. Blánquez, P. and B. Guieysse, *Continuous biodegradation of 17β-estradiol and 17α-ethynylestradiol by Trametes versicolor.* Journal of hazardous materials, 2008. **150**(2): p. 459-462.
195. Nguyen, L.N., et al., *Removal of trace organic contaminants by an MBR comprising a mixed culture of bacteria and white-rot fungi.* Bioresource technology, 2013. **148**: p. 234-241.
196. Cabana, H., A. Ahamed, and R. Leduc, *Conjugation of laccase from the white rot fungus Trametes versicolor to chitosan and its utilization for the elimination of triclosan.* Bioresource technology, 2011. **102**(2): p. 1656-1662.
197. Songulashvili, G., et al., *Immobilized Coriolopsis sp. laccase for continuous elimination and transformation of phenolic micropollutants.* Water Quality Research Journal, 2014. **49**(4): p. 328-338.
198. Asgher, M., et al., *Hyperactivation and thermostabilization of Phanerochaete chrysosporium lignin peroxidase by immobilization in xerogels.* World Journal of Microbiology and Biotechnology, 2007. **23**(4): p. 525-531.
199. Ba, S., et al., *Laccase immobilization and insolubilization: from fundamentals to applications for the elimination of emerging contaminants in wastewater treatment.* Critical reviews in biotechnology, 2013. **33**(4): p. 404-418.
200. Sheldon, R.A., *Enzyme immobilization: the quest for optimum performance.* Advanced Synthesis & Catalysis, 2007. **349**(8‐9): p. 1289-1307.
201. Datta, S., L.R. Christena, and Y.R.S. Rajaram, *Enzyme immobilization: an overview on techniques and support materials.* 3 Biotech, 2013. **3**(1): p. 1-9.
202. Garcia‐Galan, C., et al., *Potential of different enzyme immobilization strategies to improve enzyme performance.* Advanced Synthesis & Catalysis, 2011. **353**(16): p. 2885-2904.
203. DiCosimo, R., et al., *Industrial use of immobilized enzymes.* Chemical Society Reviews, 2013. **42**(15): p. 6437-6474.
204. Neifar, M., et al., *Comparative study of olive oil mill wastewater treatment using free and immobilized Coriolopsis polyzona and Pycnoporus coccineus.* Journal of Microbiology, 2012. **50**(5): p. 746-753.
205. Maryšková, M., et al., *Polyamide 6/chitosan nanofibers as support for the immobilization of Trametes versicolor laccase for the elimination of endocrine disrupting chemicals.* Enzyme and microbial technology, 2016. **89**: p. 31-38.
206. Matijošytė, I., et al., *Preparation and use of cross-linked enzyme aggregates (CLEAs) of laccases.* Journal of Molecular Catalysis B: Enzymatic, 2010. **62**(2): p. 142-148.
207. Ehlers, G. and P. Rose, *Immobilized white-rot fungal biodegradation of phenol and chlorinated phenol in trickling packed-bed reactors by employing sequencing batch operation.* Bioresource technology, 2005. **96**(11): p. 1264-1275.
208. Różalska, S., R. Szewczyk, and J. Długoński, *Biodegradation of 4-n-nonylphenol by the non-ligninolytic filamentous fungus Gliocephalotrichum simplex: a proposal of a metabolic pathway.* Journal of hazardous materials, 2010. **180**(1-3): p. 323-331.
209. Riley, R., et al., *Extensive sampling of basidiomycete genomes demonstrates inadequacy of the white-rot/brown-rot paradigm for wood decay fungi.* Proceedings of the National Academy of Sciences, 2014. **111**(27): p. 9923-9928.
210. An, H., et al., *Molecular characterization of a novel thermostable laccase PPLCC2 from the brown rot fungus Postia placenta MAD-698-R.* Electronic Journal of Biotechnology, 2015. **18**(6): p. 451-458.
211. Junghanns, C., et al., *Degradation of the xenoestrogen nonylphenol by aquatic fungi and their laccases.* Microbiology, 2005. **151**(1): p. 45-57.
212. Junghanns, C., et al., *Biochemical and molecular genetic characterisation of a novel laccase produced by the aquatic ascomycete Phoma sp. UHH 5-1-03.* Applied microbiology and biotechnology, 2009. **84**(6): p. 1095-1105.
213. Sharma, B., A.K. Dangi, and P. Shukla, *Contemporary enzyme based technologies for bioremediation: a review.* Journal of environmental management, 2018. **210**: p. 10-22.
214. Mate, D., et al., *Laboratory evolution of high-redox potential laccases.* Chemistry & biology, 2010. **17**(9): p. 1030-1041.
215. Grgic, I. and A. Perdih, *Stimulation of ligninolytic enzyme production in Phanerochaete chrysosporium by polyoxyalkanes.* Journal of applied microbiology, 2003. **94**(3): p. 360-368.
216. Mougin, C., A. Kollmann, and C. Jolivalt, *Enhanced production of laccase in the fungus Trametes versicolor by the addition of xenobiotics.* Biotechnology letters, 2002. **24**(2): p. 139-142.

217. Ramírez, D.A., et al., *Effects of different wavelengths of light on lignin peroxidase production by the white-rot fungi Phanerochaete chrysosporium grown in submerged cultures.* Bioresource technology, 2010. **101**(23): p. 9213-9220.
218. Ryu, S.-H., et al., *Enhanced lignin biodegradation by a laccase-overexpressed white-rot fungus Polyporus brumalis in the pretreatment of wood chips.* Applied biochemistry and biotechnology, 2013. **171**(6): p. 1525-1534.
219. Camarero, S., et al., *Engineering platforms for directed evolution of laccase from Pycnoporus cinnabarinus.* Appl. Environ. Microbiol., 2012. **78**(5): p. 1370-1384.
220. Kum, H., et al., *Degradation of endocrine disrupting chemicals by genetic transformants with two lignin degrading enzymes in Phlebia tremellosa.* The Journal of Microbiology, 2011. **49**(5): p. 824-827.
221. Comellas-Aragonès, M., et al., *A virus-based single-enzyme nanoreactor.* Nature nanotechnology, 2007. **2**(10): p. 635.
222. Kim, B.C., et al., *Preparation of biocatalytic nanofibres with high activity and stability via enzyme aggregate coating on polymer nanofibres.* Nanotechnology, 2005. **16**(7): p. S382.
223. Demarche, P., et al. *Laccase Production from White Rot Fungi and Immobilization on Nano Particles*. in *110th General Meeting of the American Society for Microbiology*. 2010.
224. Ansari, S.A. and Q. Husain, *Potential applications of enzymes immobilized on/in nano materials: a review.* Biotechnology advances, 2012. **30**(3): p. 512-523.
225. Liang, H., et al., *Multicopper laccase mimicking nanozymes with nucleotides as ligands.* ACS applied materials & interfaces, 2017. **9**(2): p. 1352-1360.