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Fungi for the bioremediation of pharmaceutical-derived pollutants: A bioengineering approach to water treatment

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ABSTRACT

The excessive amount of pharmaceutical compounds (PhCs) released into aquatic environments poses a risk to humans, wildlife, and environmental health. It is a serious problem that requires urgent attention. In this work, we review various PhCs detected in water treatment plants. We propose that fungi, particularly white-rot fungi (WRF), can be used for their bioremediation and describe the main mechanisms used for degrading this type of emerging pollutants; however, we also highlight the need to prospect for new fungal models. A conceptual proposal is made to develop an immobilization device containing a consortium of fungal species that can be placed in wastewater treatment plants (WWTP). We consider that this device would allow more efficient bioremediation of PhCs and address an environmental problem that remains neglected.

Introduction

The increased use of pharmaceuticals by modern societies is concomitant with their disposal into the environment. As a result of a rapid advance in medical sciences and pharmacology, numerous drugs have been developed in the last decades to treat humans and animals' frequent and rare diseases (Podolsky, 2018; Silva et al., 2015). New regulations have been put in place to release drugs based on their safety and efficacy in patients. However, efforts to understand the effects of the same drugs in the environment are still taken lightly (Halling-Sørensen et al., 1998; Jjemba, 2006). Therefore, aquatic environments are continually being exposed to a substantial load of these compounds that exceed domestic and industrial purification capabilities, representing a significant risk to wildlife and human populations. Pharmaceutical products are part of the emerging pollutants, which until now remain largely unregulated in terms of environmental health, and whose impact on the environment has not been evaluated thoroughly (Silva et al., 2015).

In this work, we review the main pharmaceutical-derived pollutants found in water bodies, the possible risks that this may represent for human health, and possibly the health of wildlife, which remains neglected. Likewise, we expose the different benefits and potential of fungi, particularly White-Rot Fungi (WRF), for the bioremediation of these emerging pollutants. We highlight the need to prospect new fungal models and present some ideas on how these could be used to bioengineering wastewater treatment plants, particularly in the active sludge phase.

Pharmaceutical-derived pollutants in water

Most pharmaceuticals are hydrophilic and biologically active compounds designed to be easily absorbed by the body and prevent their degradation before it has curative effects (Halling-Sørensen et al., 1998; Silva et al., 2015). These compounds are precisely intended to affect the biological systems' functions biochemically or physiologically of humans or animals, while their activity persists even outside the body (Jjemba, 2006). Depending on the substance, it can be excreted in urine or feces as the substance without alteration or in a mixture of metabolites related to the primary compound known as pharmaceutical compounds (PhCs) (Halling-Sørensen et al., 1998; Radjenović et al., 2009). The residual metabolites of partial degradation can, in some cases, become more toxic or biologically active than the drug itself (Richardson, 2017; Wu et al., 2009). It has been reported for antibiotics that 50%–90% administered to humans or animals are excreted as a mixture of compounds and metabolites of the parent compound (Kümmerer, 2009a). Sulfonamide antibiotics, one of the most widely used antibiotics in the world, and their acetylated metabolites have been detected in different environmental samples, as in influent and effluent wastewater samples, rivers, and sediments (Díaz-Cruz et al., 2008; García-Galán et al., 2012; Yuan et al., 2019; Cui et al., 2020). The concentration of

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acetyl sulfamethoxazole, a derivative of the parent molecule, for example, has reached levels up to 1.10 ng /g in the WWTP sludge (Cui et al., 2020). Metabolites of the antibiotics oxytetracycline and clarithromycin, have shown evident toxicity to fish, microcrustacean, green alga, cyanobacterium (Baumann et al., 2015), and rats (Han et al., 2016).

The presence of pharmaceutical-derived emerging pollutants in waters can represent a long-term risk to human health and aquatic ecosystems, even under low concentration levels (Tran et al., 2019). Table 1 shows a list of pharmacological compounds found in effluent water from treatment plants and present in surface water. In brief, levels from the order of ng/L to 373 μg/L of different drugs have been detected in the influent water of wastewater treatment plants (WWTP) (Köck-Schulmeyer et al., 2011; Verlicchi et al., 2012). Anti-hypertensive drugs, antibiotics, diuretics, beta-blockers, and anti-inflammatory drugs were the ones with the highest concentrations in the influent water to the WWTP, considered the most consumed drugs (Köck-Schulmeyer et al., 2011; Verlicchi et al., 2012). More specifically, the drugs ibuprofen, acetaminophen (Rosal et al., 2010), tramadol, carbamazepine (Richardson, 2017), and naxopren (Al Aukidy et al., 2012) presented the highest concentrations in untreated wastewater (Verlicchi et al., 2012). In addition, concentrations of up to ~30 μg/L have been shown for antibiotics such as ofloxacin, roxithromycin, ciprofloxacin, and beta-lactams (Kümmerer, 2009a). In a study in the WWTP of Costa Rica, drug concentrations in effluent waters ranged from 0.10 to 66.9 μg /L, where naxoprene and gemfibrozil, anti-inflammatory and a lipid regulator, respectively, were the most common pharmaceutical pollutants (Ramírez-Morales et al., 2020).

In general, WWTPs are not designed to treat emerging pollutants, so the concentrations of pharmaceutical compounds (PhCs) in the effluents are similar to those of the influents. Some common PhCs found in high concentrations in WWTPs effluents include analgesics (i.e., tramadol, dipiron, and ibuprofen), psychiatric drugs (i.e.carbamazepine), antidiabetics, and anti-inflammatories in ranges from 0.001 μg/L to 57 μg (Radjenović et al., 2009; Sgroi et al., 2017). Some antibiotics such as ciprofloxacin, erythromycin, roxithromycin, and ofloxacin have been detected in concentrations up to 6.7 μg/L. (Verlicchi et al., 2012). The detection of other drugs such as anti-hypertensives, beta-blockers, diuretics, and lipid regulators in effluent waters, also shows that WWTPs are deficient in the degradation of these compounds, whose effects on aquatic ecosystems remain poorly studied (Gogoi et al., 2018; Jjemba, 2006; Petrović et al., 2003). It is important to mention that the analysis and detection of some PhCs may represent an analytical challenge due to their low concentrations and the wide diversity of chemical properties, which would imply more PhCs in the environment than those determined with current techniques (Richardson, 2017).

Due to their chemical-physical properties, such as the low degradation rate and high solubility, PhCs can penetrate the filtration stages of conventional treatments of the WWTP. A large proportion can escape the elimination and enter the aquatic environment. Emerging pollutants of pharmaceutical origin have been detected in surface waters (Tran et al., 2018), groundwaters (Giger et al., 2003), and drinking waters (Mahmood et al., 2019; Rodríguez-Rodríguez et al., 2013). These compounds can pose a serious risk to human health and the environment (Petrović et al., 2003). Aquatic life can be susceptible to PhCs. For example, it has been shown that bacteria's growth is substantially repressed by concentrations greater than 150 μg/mL of ibuprofen (Elvers & Wright, 1995). The presence of antibiotics in waters has been shown to affect the growth of green algae, crustaceans, and copepods (Gaw et al., 2014), and polychaetes (Fonseca et al., 2018). The generation of resistance to antibiotics represents a challenge for environmental, human, and veterinary health (Halling-Sørensen et al., 1998; Kümmerer, 2009b).

Antibiotics as emerging pollutants represent a significant concern due to their adverse effects on the terrestrial and aquatic ecosystems, mainly in the affectation of bacteria, fungi, and microalgae. Bacteria have a great capacity to adapt to environmental changes and surviving unfavorable conditions due to the rapid generation time and ability to acquire new traits through horizontal gene transfer (Kümmerer, 2009b; Tran et al., 2018; Von Wintersdorff et al., 2016). Underexposure to antibiotics, bacteria have been able to generate resistances, resulting in deficiencies to effectively treat bacterial infections of humans and animals (Kümmerer, 2003).

Antineoplastic drugs to treat cancer pose a concern due to their acute effects in low concentrations. They can generate genetic changes, having an accumulative impact over long periods that can lead to profound ecological consequences (Daughton & Ternes, 1999). Two types of antineoplastic therapies are currently used, cytotoxic drugs, which generally act on the function and structure of DNA, and endocrine drugs, which function as hormone disruptors (Azuma, 2018; Johnson et al., 2008). These can affect the normal growth, survival, and reproduction rate of several aquatic non-target species (reviewed in Besse et al., 2012). These effects can acutely perpetuate in living organisms (Gros et al., 2010), but for the most part, they represent a potential chronic ecological risk and cumulative ecotoxicity in the presence of a mixture of a wide variety of drugs at very low concentrations (Cleuvers, 2003, 2004; Richardson, 2017; Verlicchi et al., 2012). Therefore, the assessment of the ecotoxicology of PhCs require to be realistic to their characteristics and to the fact that the actual effects are observed in the long term (life cycle cytotoxic assessment) and they transient even to the population scale, not limiting to individuals physiology (Besse et al., 2012; Nassour et al., 2020).

Routes of emerging pollutants to the environment

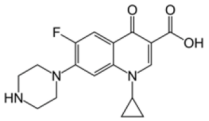
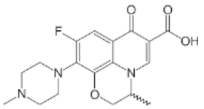
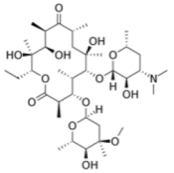
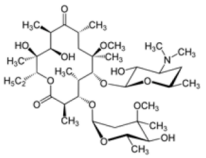
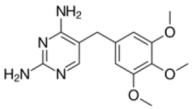
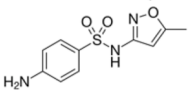
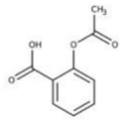
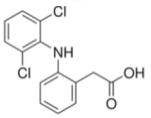
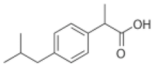
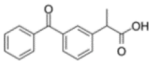
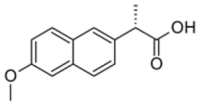
When a drug is consumed, it is internalized and absorbed in the human or animal body; its availability will depend on the molecule's intrinsic characteristics, such as solubility, connectivity, electronic nature, size, and shape (Jjemba, 2006). Depending on how the drug dissolves and is metabolized in the animal body, the parental molecule or secondary compounds will be excreted through feces or urine from homes or hospitals (Kümmerer, 2003) or by direct disposal of the unused medications (Kümmerer et al., 2016). These waters are directed to wastewater treatment plants that, once treated, are released to surface waters (Gogoi et al., 2018; Jjemba, 2006).

In turn, the excretion of animals such as livestock is used as fertilizer, with the PhCs being able to contaminate the soil (Biel-Maeso et al., 2018) and groundwater (Halling-Sørensen et al., 1998; Wohde et al., 2016) or been treated in WWTP, with the last stop of the surface waters (Gogoi et al., 2018). Also, many industries deposit their wastewater directly into surface waters or wastewater treatment plants (Fick et al., 2009; Giger et al., 2003). Groundwater and surface water are used after several more water treatments to supply houses, hospitals, and industries with drinking water (Giger et al., 2003; Petrović et al., 2003).

Thus, WWTPs are the main wastewater receivers, essentially consisting of consecutive stages where the water is progressively purified. As the first barrier against influent water, WWTPs include bar grids (pretreatment) that aim to remove oversized materials and protect the plant from clogs (Spellman, 2013). Along with this comes the second step of homogenization of the sewage water, where the non-organic matter is removed in a grit chamber combined with aeration. Typically, it is followed by a primary treatment (settling velocity), where is removed settleable organic (i.e., feces) and floatable solids (i.e., soap foam); as a result, the effluent is expected to present only small organic matter (<10 μm) (Spellman, 2013). In this primary homogenization treatment, it is possible to generate the by-product of floating oils that, in turn, store pharmaceutical products with high $\log K_{ow}$ values, such as gemfibrozil, phenacetin, and diclofenac (Bo et al., 2015). Even so, the concentrations that can be stored in this hydrophobic phase of the treatment are low, with most of the initial concentrations continuing the journey to sequential treatment.

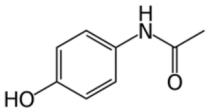
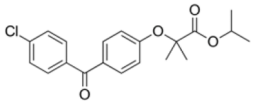
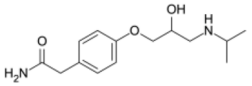
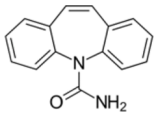
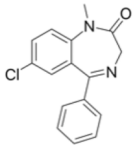
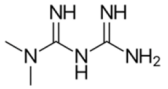
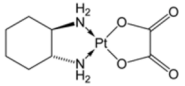
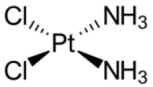
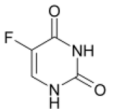
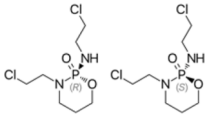
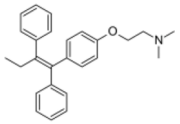
This effluent water of the primary treatment enters the secondary

Table 1
Occurrence of pharmaceutical compounds in effluent water from wastewater treatment plants and environmental surface water bodies.

Type	Compound	Structure	Environmental Occurrence (ng/L)	Site	Reference
Antibiotics	Ciprofloxacin		0 – 5692Nd – 26.2	Effluent WWTPSurface water (river)	(Mahmood et al., 2019) (K'oreje et al., 2016) (Biel-Maeso et al., 2018) (Rosal et al., 2010)(Zuccato et al., 2005)
	Levofloxacin		0 - 177	Effluent WWTP	(Mahmood et al., 2019) (K'oreje et al., 2016)
	Ofloxacin		37 – 165166 - 68	Effluent WWTPSurface water (river)	(Biel-Maeso et al., 2018)(Christian et al., 2003) (Alexy et al., 2006) (Radjenović et al., 2009)
	Erythromycin		18 – 76095 - 109	Effluent WWTPSurface water (river)	(Biel-Maeso et al., 2018)(Rosal et al., 2010)(Christian et al., 2003) (Radjenović et al., 2009)
	Clarithromycin		7 – 7640 62 - 103	Effluent WWTPSurface water (river)	(Biel-Maeso et al., 2018) (Christian et al., 2003)(Alexy et al., 2006) (Sgroi et al., 2017)
	Sulfamethoxazole		26 – 633100 - 203	Effluent WWTPSurface water (river)	(Biel-Maeso et al., 2018; K'oreje et al., 2016) (Papageorgiou et al., 2019) (Rosal et al., 2010)(Christian et al., 2003)
Anti-inflammatoryAnalgesics	Acetylsalicylic acid		0.3 -1.4 23 -419	Drinking waterEffluent WWTP	(Biel-Maeso et al., 2018; Rabiet et al., 2006))
	Diclofenac		1.4 - 2.5 1.4 - 33.2 6 - 1020	Drinking waterSurface water (river) Effluent WWTP	(Biel-Maeso et al., 2018; Rabiet et al., 2006) (Radjenović et al., 2009)(Reviewed in Vieno & Sillanpää, 2014)
	Ibuprofen		0.2 - 0.6 0.3 - 4.518 - 715	Drinking waterSurface water (river) Effluent WWTP	(Biel-Maeso et al., 2018) (Rabiet et al., 2006) (K'oreje et al., 2016)
	Ketoprofen		0.6 - 3.02.8 - 14.522 - 5480	Drinking waterSurface water (river) Effluent WWTP	(Biel-Maeso et al., 2018; Rabiet et al., 2006)
	Naproxen		0.1 - 0.27.2 - 9.140 - 2208	Drinking waterSurface water (river) Effluent WWTP	(Biel-Maeso et al., 2018; Rabiet et al., 2006) (Rosal et al., 2010)

(*continued on next page*)

Table 1 (*continued*)

Type	Compound	Structure	Environmental Occurrence (ng/L)	Site	Reference
	Paracetamol (acetaminophen)		8.3 - 42.510.6 - 72.317 – 113000 – 31,614	Drinking waterSurface water (river) Effluent waterHospital effluent WWTP	(Biel-Maeso et al., 2018; Rabiet et al., 2006) (K'oreje et al., 2016)(Papageorgiou et al., 2019)
Lipid regulators/Anti-hypertensives	Fenofibrate		1-146	Effluent WWTP	(Biel-Maeso et al., 2018)
	Atenolol		134 - 2438	Effluent WWTP	(Biel-Maeso et al., 2018) (Papageorgiou et al., 2019) (Rosal et al., 2010) (Radjenović et al., 2009)
Psychiatric drugs	Carbamazepine		13.9 - 43.2 23.6 - 56.3 69 - 293	Drinking waterSurface water (river) Effluent WWTP	(Rabiet et al., 2006) (K'oreje et al., 2016) (Al Aukidy et al., 2012)
	Diazepam		7 – 241.08 - 35.1	Effluent WWTPSurface water (river)	(K'oreje et al., 2016) (Al Aukidy et al., 2012) (López-Serna et al., 2013)
Antidiabetic	Metformin		0 - 1159	Hospital effluent WWTP	(Papageorgiou et al., 2019)
Antineoplastic	Oxaliplatin		0 - 0.499	Predicted effluent WWTP	(Rowney et al., 2009)
	Cisplatin		0 – 0.601	Predicted effluent WWTP	(Rowney et al., 2009)
	5-fluorouracil (5-FU)		8600 – 124000Nd - 122000	Hospital Influent WWTPHospital Effluent WWTP	(Mahnik et al., 2007) (Isidori et al., 2016) (Wormington et al., 2020)
	Ifosfamide		6 - 86200	Hospital effluent WWTP	(Gómez-Canela et al., 2014)
	Tamoxifen		Nd – 17025 - 38	Hospital effluent WWTPSurface water (river)	(Ferrando-Climent et al., 2014) (Isidori et al., 2016)

Nd=No detection

treatment known as the aeration basin. At this stage, microorganisms such as bacteria and fungi play a fundamental role, promoting organic matter degradation (Abdel-Raouf et al., 2019; Akratos, 2016; Stott, 2003). The set of microorganisms internalized in the aeration basin is known as the activated sludge (Seviour, 2010). Indeed, high biodegradation rates of endocrine disruptors (Stasinakis et al., 2010) and antibiotics (Li & Zhang, 2010; Yang et al., 2012) have been evidenced in activated sludge chamber. Nonetheless, the massive amounts of these daily pollutants exceed the current capacities of the activated sludge. The activated sludge mechanism created more than a century ago faces another panorama of pollution, not capable of a substantial degradation. Consequently, xenobiotics' contaminants reach the environment (Radjenović et al., 2009; Scholz, 2006). The induction of bubbles favors the biodegradation in the secondary treatment to obtain high oxygen concentrations, which favor the microorganisms' aerobic metabolism. In this clarifier, some PhCs sufficiently labile, such as acetaminophen, lower their concentrations due to aerobic biodegradation (from 186 to 0.51 μg/L), no longer changing to the final effluent water (Brown & Wong, 2018). However, there are other PhCs, such as propranolol and thyroxine, where their concentrations are not altered throughout the treatments (Brown & Wong, 2018). Finally, the residual activated sludge is returned, and the treated water is processed by a tertiary microorganism disinfection treatment with i.e., chlorine, ozone, and UV (Bourgin et al., 2018). This multi-step process is typically found in all treatment plants, with variation and implementation of other novels additional steps (Krzeminski et al., 2017; Liu et al., 2020; Neoh et al., 2016). Threateningly, the primary source of drugs to the environment is from WWTP since they are not designed to degrade these emerging pollutants (Franquet-Griell et al., 2015; Gros et al., 2010; Petrović et al., 2003). They were evidencing how pharmaceutical pollutants represent an engineering problem in the design and operation of WWTP in the current global situation, which can lead to significant ecological, environmental, and health problems.

Fungi to the rescue

Fungi are recognized as the microorganisms responsible for the degradation of most organic compounds in the environment. Since the 1980s, fungi belonging particularly to the WRF have been used in water and soil bioremediation processes (Rodríguez-Rodríguez et al., 2013). The WRF (mainly basidiomycetes) are composed of an eco-physiological group of fungi capable of degrading lignin (Hale & Eaton, 1985; Rodríguez-Rodríguez et al., 2013). This fungal group presents enzymatic machinery recognized as lignin modifying enzymes (LME) in charge of lignin degradation and wood decomposition (Rodríguez-Rodríguez et al., 2013). Due to the low specificity of this enzymatic machinery, other targets, including a large number of contaminating compounds such as PhCs and antibiotics, can be degraded (Asgher et al., 2008; Marco-Urrea et al., 2009, 2010; Cruz-Morató et al., 2013, Cruz-Morató et al., 2014).

The diversity and non-specificity of WRF enzymes make them potential tools for the bioremediation of drugs and antibiotics (Figure 1) (Ellouze & Sayadi, 2016; Haroune et al., 2017; Naghdi et al., 2018; Ryan et al., 2007). The use of fungi to treat pollutants has a series of advantages over other physical and chemical mechanisms, such as the high effectiveness, low cost, and environmentally friendly alternative (Tomasini & Hugo León-Santiesteban, 2019). In addition, bioremediation with fungi has advantages over bioremediation with bacteria due to the diversity of processes and degrading enzymatic capacities and their ability to function under broad pH conditions (Khursheed & Kazmi, 2011; Tomasini & Hugo León-Santiesteban, 2019). Furthermore, the hyphal morphology of WRF can promote water purification through biosorption, where PhCs and other chemicals adhere to their surface or can be internalized in the cell, being retained and not carried in the water (Kumar & Min, 2011; Lu et al., 2016).

The biodegradation of PhCs by the WRF fungi is mediated predominantly by the activity of LME, which chemically modify xenobiotic

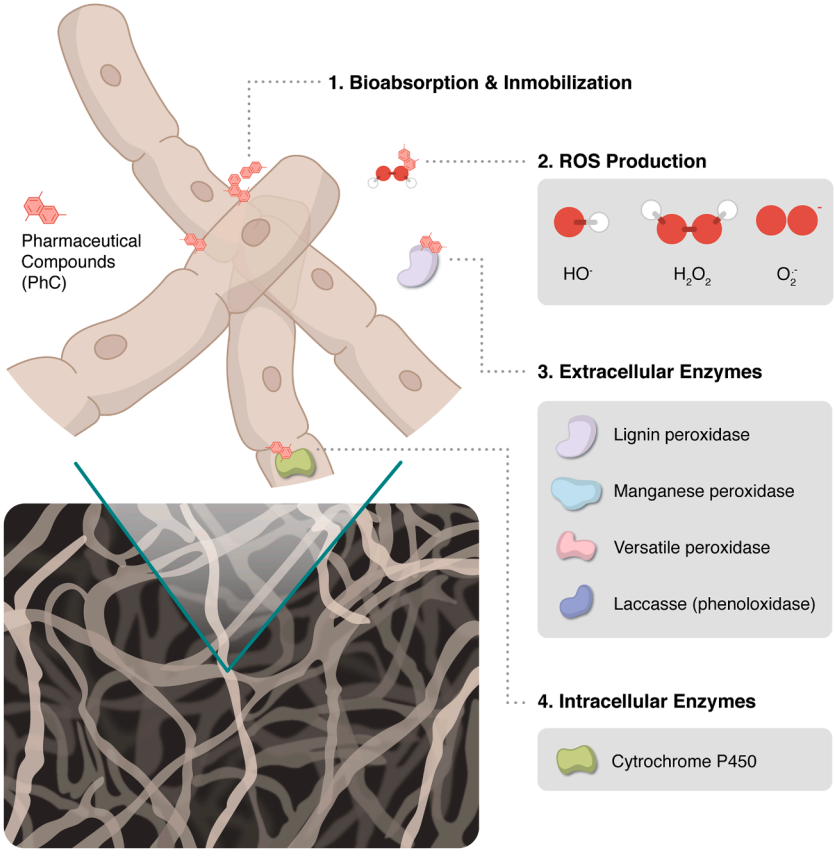


Fig. 1. Degradation capacities of fungi. Scheme of the four main mechanisms that aquatic fungi can use for the bioremediation of pharmaceutical compounds (PhCs) in water treatment plants. 1) Bio absorption and immobilization of the PhCs due to their hyphal morphology. 2) Reactive oxygen species production, such as hydrogen peroxide (H_2O_2), superoxide anion radicals (O_2^-.), and hydroxyl radical (OH^-). 3) Extracellular enzymatic machinery, peroxidase, and phenoloxidase enzymes. 4) Intracellular enzymatic machinery (Cytochrome P450 complex). All these mechanisms can operate either independently or synergistically.

compounds involving the action of oxidoreductase enzymes (Dhouib et al., 2006; Gernaey et al., 2004; Pointing, 2001). This group of highly oxidative enzymes can function intra or extracellularly, therefore, not requiring the compound's complex internalization and the respective possible cellular toxicity (Naghdi et al., 2018; Carlos E. Rodríguez-Rodríguez et al., 2014). Among the enzymes secreted by fungi and that function extracellularly are laccases and peroxidases (i.e., lignin peroxidase, manganese dependent peroxidase, versatile peroxidase) (Lu et al., 2016; C. E. Rodríguez-Rodríguez et al., 2013). Both laccases and peroxidases are nonspecific glycoprotein enzymes that can catalyze the oxidation of aromatic compounds such as phenols, which are constituents in most drugs (Asgher et al., 2008; Ellouze & Sayadi, 2016; Naghdi et al., 2018).

The oxidation processes due to the action of LME can lead to the formation of radicals and reactive oxygen species, which act as oxidative mediators to oxidize different compounds to a greater degree (Cañas & Camarero, 2010; Ijoma & Tekere, 2017; Rodríguez-Rodríguez et al., 2014). For example, it has been demonstrated that the production of hydrogen peroxide (H_2O_2) and superoxide anion radicals ($O_2^{-\cdot}$), which can lead to hydroxyl radical (OH^-) by the Fenton reaction, can be enhanced by the dismutation and laccase oxidation of hydroquinone and aromatic aldehyde by *Pleurotus eryngii* (Gómez-Toribio et al., 2009; Guillén et al., 2000). In addition, the cytochrome P450 complex corresponds to the most important intracellular enzyme systems, consisting of a superfamily of enzymes that function as monooxygenases with a heme group as a cofactor. These enzymes catalyze dealkinalization, deamination, dehalogenation, hydroxylation processes, altering the compound and leading to its possible mineralization (Asif et al., 2017; Rodríguez-Rodríguez et al., 2014; Yang et al., 2013). This system is generally coupled in the synergistic act of degradation with the other extracellular enzymatic machinery.

Trametes versicolor (Rodriguez-Rodriguez et al., 2011; Rodríguez-Rodríguez et al., 2010), *Phanerochaete chrysosporium* (Huang et al., 2017) and *Phlebia tremellosa* (Kum et al., 2011) are some of the most common WRF when it comes to bioremediation of contaminants (Marco-Urrea et al., 2010; Rodríguez-Rodríguez et al., 2010). *T. versicolor* is one of the most used fungi, since it has laccase and lignin peroxidase activity (dependent on Mn), and also the P450 complex (Marco-Urrea et al., 2010). The functional versatility of this species makes it very useful for the degradation of xenobiotic compounds (Asif et al., 2017; Rodríguez-Rodríguez et al., 2019; Ryan et al., 2007). It has been shown that *T. versicolor* can effectively degrade the drugs naxopren and carbamazepin (Rodríguez-Rodríguez et al., 2010) and ibuprofen (Marco-Urrea et al., 2009) derived from wastewater. Also, anti-depressants such as citalopram and fluoxetine, detected in effluenst of WWTPs (Kwon & Armbrust, 2006), were highly degraded by incubation of *Bjerkandera adusta* and *P. chrysosporium* (Rodarte-Morales et al., 2011), compared with alternative degradation strategies (Kwon & Armbrust, 2005). Other drugs such as diclofenac, ibuprofen and naproxen are rapidly degraded by WRF, reaching 50% degradation levels after 7 days of incubation (Rodarte-Morales et al., 2011).

Due to their morphological and biochemical characteristics, fungi represent today a biotechnological option to solve water treatment's significant challenges. Although many efforts have been made to identify fungi in WWTP, it is necessary to prospect for more fungi to face the reality of water contamination (Cruz del Álamo et al., 2020; Li et al., 2016; Liang et al., 2012). Considering that PhCs form complex and diverse mixtures (Lu et al., 2016), the use of fungal cocktails could bring a greater diversity of biodegradative and biosorption capacities for the greater efficiency in the degradation of PhCs in polluted waters (Mishra & Malik, 2014; Rahman et al., 2014). Further work is still needed to identify novel fungal species with the enzymatic capabilities to degrade these compounds of pharmacological origin and capable of proliferating and function in wastewater treatment plants.

Conceptual proposal for the use of fungi in WWTPs

The design of WWTPs includes various physical and chemical processes and a secondary biological treatment performed in the activated sludge unit (Abdel-Raouf et al., 2019; Anastasi et al., 2012). Some WRF are present in the different phases yet being minimally representative of the total abundance of microorganisms (Dhouib et al., 2006; Gernaey et al., 2004). We propose that more significant success in the degradation of PhCs could be achieved by increasing the quantity and diversity of fungi in the activated sludge phase.

In this sense, it would be very convenient to have a device that allows the fungi to be confined within a porous matrix in the secondary treatment phase (Fig. 2). This would increase efficiency in the degradation of PhCs, which are not usually targets in conventional plants (Mao & Guan, 2016; Radjenovic et al., 2007). The use of fungi for the degradation of PhCs and similar compounds is not necessarily a new idea (Rodríguez-Rodríguez et al., 2014; Tortella et al., 2015; Gullotto et al., 2015; Spennati et al., 2020), however, the bioprospecting of new strains, the use of fungal cocktails, as well as their immobilization and disposal in a removable device represents a possible innovation of high value for the treatment of these pollutants.

In general, the following steps compose a roadmap for developing the device: 1) the bioprospecting of more extensive diversity of fungi in several WWTPs. This step includes the isolation and characterization of novel strains, the determination of LME, and the evaluation of degradation rates of PhCs *in vitro*. 2) selecting a fungal cocktail containing various species with the four main groups of enzymatic activities. It is crucial to consider the optimization of the fungal cocktail through compatibility-antagonism tests. 3) The establishment of the fermentation process in the solid phase and selecting a suitable matrix for its production. 4) The design and construction of the container device. 5) Evaluation tests of the degradation rate of PhCs in WWTPs, replacement frequency, and optimization of the system.

In this regard, a consortium of fungi can promote a complementary and synergic effect between the different mechanisms used by each organism, and thus for the metabolites produced from the degradative interactions with the parental molecule (Olicón-Hernández et al., 2017; Papageorgiou et al., 2019). Previous works have shown that fungal consortia present higher degradation efficiencies of several xenobiotics in WWTPs (Gullotto et al., 2015; Spennati et al., 2020; Talukdar et al., 2020). In particular, a consortium of fungi composed of *Aspergillus niger, Mucor circinelloides, Trichoderma longibrachiatum, Trametes polyzona, and Rhizopus microspo*res have shown to actively degrade PhCs such as carbamazepine, diclofenac and ibuprofen, and their by-products (Kasonga et al., 2020).

It is crucial that the fungal cocktail can be inoculated directly into a solid matrix embedded in a simple culture medium, where the fungi can grow and be immobilized. After few days of growth, when hyphae are anchored to the matrix, the unit can be incorporated into the device for use in WWTP. The solid phase has proved very successful in the bioremediation process of emerging pollutants in WWTPs (Carballa et al., 2007; Rahman et al., 2014). In addition, due to the saprophytic nature of fungi, their nutritional requirements can be easily achieved in the same degradation medium, which is an inexpensive treatment method (Fakhru'l-Razi & Molla, 2007; Lu et al., 2016). However, it is essential to consider growth conditions such as pH, temperature, ionic strength, and C / N ratio to favor the growth of fungi over bacteria (Lu et al., 2016; Badia-Fabregat et al., 2016; Espinosa-Ortiz et al., 2016) and to enhance enzymatic activities and the biosorption processes (Gao et al., 2010; Mir-Tutusaus et al., 2018). In this sense, the advantage of bioprospecting fungi in WWTPs is that the organisms are already adapted to the conditions they will operate in the future.

The immobilization of the fungal biomass is a crucial aspect to consider, since the dispersed growth of the mycelium can cause operational difficulties (Mir-Tutusaus et al., 2018; Negi et al., 2020). Therefore, the immobilization of fungi in e.g., interweaved hyphal aggregates,

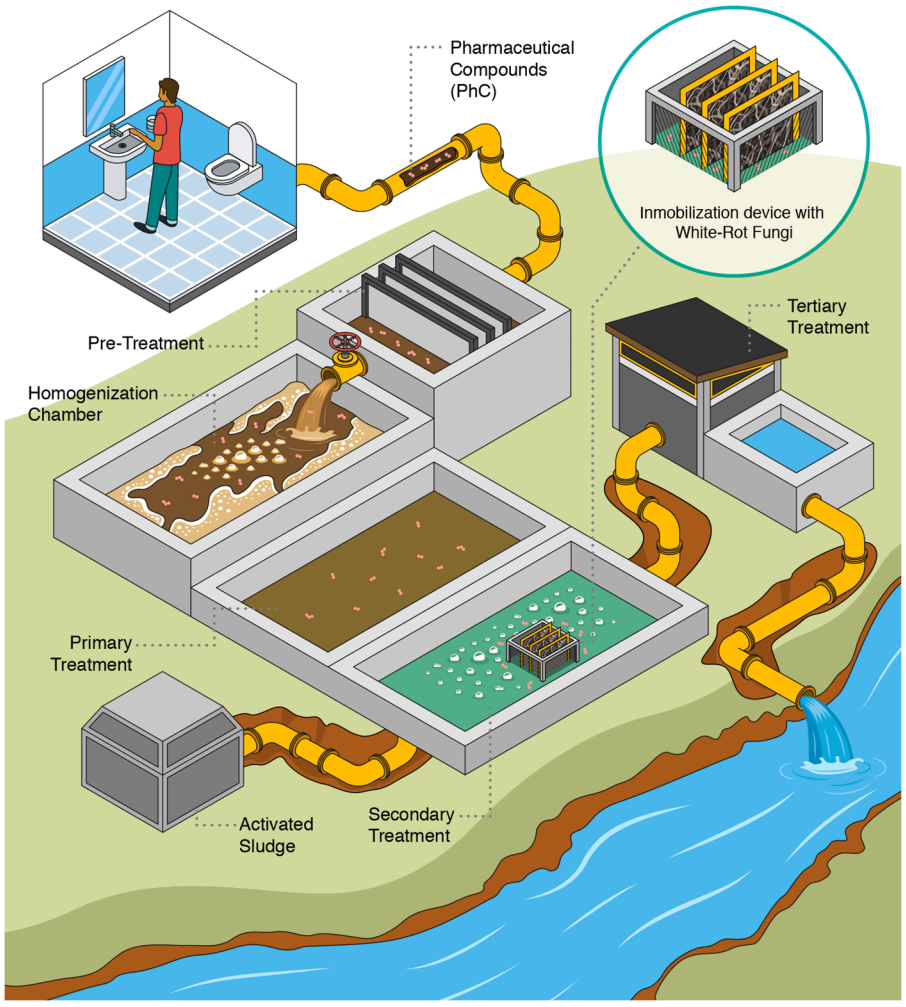


Fig. 2. Fungal bioremediation strategy. Representation of the immobilization device of a consortium of fungal species implemented in the secondary treatment of wastewater treatment plants. A possible route of pharmaceutical compounds is represented in the diagram.

ranging from micrometers to millimeters, is suggested for the effective operation of the device (Espinosa-Ortiz et al., 2016). For example, it has been shown that fungi in immobilized solids have increased the degradative efficiency of compounds such as analgesics, psychiatric drugs, lipid regulators, and antibiotics (Fakhru'l-Razi & Molla, 2007; Rodriguez-Rodriguez et al., 2011; Bernats & Juhna, 2018; del Álamo et al., 2018). *P. chrysosporium* in wood chips has shown high removal efficiencies for carbamazepine and naproxen, with an increase of 28% and 4%, respectively (X. Li et al., 2015). Immobilized *T. versicolor* on sorghum has also been used to remove humic substances derivated from industrial wastewater (Zahmatkesh et al., 2017). Also, *T. versicolor* biofilm in K1 carriers have shown 99.9% removal of diclofenac after 3 hours of incubation in non-sterile residual water (Dalecka et al., 2020).

An ideal immobilization matrix should have an open and porous structure for immediate contact of immobilized cells with the aqueous medium containing the PhCs. It should be resistant, stable for long periods with repeated uses, of easy handling, and low cost (Saeed & Iqbal, 2013). Some examples of immobilization matrices include gel matrices, silica-alginate-fungus biocomposites (Carabajal et al., 2016; Dzionek et al., 2016), and the use of the Loofa sponge (Iqbal & Edyvean, 2007; Iqbal et al., 2005). Also, the permeable reactive bio-barriers has shown success in the biodegradation of complex compounds (Simon & Meggyes, 2001). In these bio-barriers, a porous support is used to grow the filamentous fungi, forming a biofilm capable of interacting with the environment. For example, *Trichoderma longibrachiatum* immobilized with nylon sponge in reactive bio-barriers has shown great efficiency in removing polycyclic aromatic hydrocarbon compounds, which are frequently found in the chemical structures of PhCs (Cobas et al., 2013).

Finally, as a result of this work, we provide the conceptual and theoretical bases for developing a device for the bioremediation of PhCs in WWTPs. This device contains a porous matrix where a consortium of different species of fungi and with different enzymatic activities would be immobilized, and that can be installed in the secondary treatment phase. The device promotes fungal growth and activity by generating a defined space for biofilms while allowing the passage of influent water. In addition, it can be easily removed and replaced. We consider the development of this technology feasible, aimed at treating emerging contaminants of pharmacological origin.

Conclusion and perspectives

In conclusion, white-rot fungi can be considered a very useful tool for the bioremediation of emerging contaminants of pharmaceutical origin. Their ability to degrade wide variety of recalcitrant molecules and their easy handling make them excellent biological agents to include in wastewater treatment processes. We proposed to identify novel fungi with enzymatic capacities that can degrade different types of PhCs. A more considerable diversity of fungi and associated mechanisms would allow the development of new bioinoculants (fungal cocktails) for WWTPs, which could be confined in a particular removable device placed, for example, in the secondary treatment phase. A device of this nature would allow more efficient bioremediation of emerging

pollutants and a more efficient global water treatment. We present a good example of the need for greater integration between microbiology, biotechnology, and engineering to address a serious environmental problem that remains neglected.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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