Can white-rot fungi be a real wastewater treatment alternative for organic micropollutants removal? A review

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Abstract

Micropollutants are a diverse group of compounds that are detected at trace concentrations and may have a negative effect on the environment and/or human health. Most of them are unregulated contaminants, although they have raised a concern in the scientific and global community and future regulation might be written in the near future. Several approaches have been tested to remove micropollutants from wastewater streams. In this manuscript, a focus is placed in reactor biological treatments that use white-rot fungi. A critical review of white-rot fungal-based technologies for micropollutant removal from wastewater has been conducted, several capabilities and limitations of such approaches have been identified and a range of

solutions to overcome most of the limitations have been reviewed and/or proposed. Overall, this review argues that white-rot fungal reactors could be an efficient technology to remove micropollutants from specific wastewater streams.

Contents

1. Overview

Micropollutants can be defined as substances that may be bioaccumulative, persistent and toxic and may have a negative effect on the environment and/or human health, even at trace concentrations. This diverse group contains, but is not restricted to: pharmaceutically active compounds (PhACs), personal care products, endocrine disruptors, pesticides and industrial chemicals. Several authors have referred to them also as emerging contaminants, preferably termed *contaminants of emerging concern (Sauvé and Desrosiers, 2014)*. Most of them are unregulated pollutants, although future regulation might be written depending on research (Verlicchi et al., 2010). These contaminants remain biologically active even at concentrations of few ng·L^{-1}, may be accumulated through the food chain and can have negative effects on the environment, fauna and human health. The World Health Organization (2016), for example, raised concern on the development of antibiotic resistance on target bacteria due to exposure to non-lethal concentrations of antibiotics.

The origin of these pollutants is diverse: from industrial waste streams to human-excreted metabolized and non-metabolized medicaments. Typically such compounds enter the environment through municipal or industrial effluent, but they are not completely removed in wastewater treatment plants (WWTPs), which are mainly designed for removing macropollutants such as organic matter, nutrients and suspended solids (Evgenidou et al., 2015; Frédéric and Yves, 2014; Kaiser et al., 2014). In fact, micropollutants have been found in surface water, groundwater, drinking water and sewage (Dai et al., 2015).

Answering to these concerns, the scientific community has devoted extensive research into mechanisms to degrade, transform and /or remove micropollutants from wastewater. Among the possible treatments, white-rot fungi (WRF) are regarded as an effective possibility due to their

capacity to transform most of the compounds studied so far thanks to their versatile enzymatic machinery.

This manuscript reviews the bioremediation capabilities of WRF and the success examples of application with different types of micropollutants, primarily focusing on continuous treatments. Some drawbacks of the technology, largely related to the non-sterility of wastewater, are analyzed and solutions discussed.

2. Bioremediation capabilities of white-rot fungi

2.1. White-rot fungi and their enzymatic machinery

The term *white-rot fungi* is not a taxonomical grouping but rather a collection of fungal species that are able to degrade lignin (Dashtban et al., 2010). WRF are mainly basidiomycetes and some relevant species include *Pleurotus ostreatus, Phanerochaete chrysosporium, Trametes versicolor, Ganoderma lucidum* and *Irpex lacteus*.

In the environment, WRF efficiently break down lignin to release the more easily metabolized carbohydrates hemicellulose and cellulose –oxidation of lignin yields no net energy gain (Leonowicz et al., 1999). To do so, they rely on a combination of extracellular ligninolytic enzymes, organic acids, mediators and accessory enzymes. A bold feature of this enzymatic machinery is its non-specificity, due to its action via the generation of radicals. This property makes the extracellular white-rot fungal enzymes capable of transforming a wide range of organic molecules, including micropollutants.

White-rot fungi secrete lignin modifying enzymes (LMEs) and other compounds for lignin degradation. LMEs include laccase, lignin peroxidase (LiP), manganese peroxidase (MnP) and versatile peroxidase (VP). The main difference between laccases and peroxidases is that the

94 former uses molecular oxygen whilst the others use hydrogen peroxide (H_2O_2) as electron
95 acceptor.

96 Enzyme characteristics and their action mechanisms are widely described previously
97 (Camarero et al., 1999; Harvey et al., 1992; Hofrichter, 2002; Jones and Solomon, 2015; Reddy,
98 1995; Ruiz-Duenas et al., 1999), as well as, their biotechnological applications (Bogan and
99 Lamar, 1996; Rodríguez Couto et al., 2006; Van Driessel and Christov, 2001).

100 The composition of the growth medium and culture conditions highly condition the
101 production of ligninolytic enzymes (Nerud and Misurcova, 1996). In addition to LMEs, WRF
102 can also produce and secrete redox mediators that act as vehicles for electron transfer and further
103 expand the range of substrate for the ligninolytic enzymes (Cañas and Camarero, 2010; Marco-
104 Urrea et al., 2010b; Morozova et al., 2007; Pointing, 2001). In spite of the extraordinary
105 extracellular enzymatic system of WRF, it is not the only responsible of microcontaminant
106 degradation. Cytochrome P450 constitutes a superfamily of intracellular heme-containing
107 monooxygenases ubiquitous in all biological kingdoms. In fungi, they play a role in
108 housekeeping biochemical reactions, detoxification of xenobiotics and adaptation to hostile
109 ecological niches (Durairaj et al., 2016). The involvement of cytochrome P450 in degradation of
110 several micropollutants has been largely described: trinitrotoluene (Spiker et al., 1992),
111 polycyclic aromatic hydrocarbons (Yadav and Reddy, 1993), the dye malachite green (Cha et
112 al., 2001), the organochlorine compounds polychlorinated dibenzodioxins and dichloro-
113 diphenyl-trichloroethane (Kamei and Kondo, 2005; Xiao et al., 2011), carbamazepine and
114 clofibric acid (Marco-Urrea et al., 2009), ketoprofen (Marco-Urrea et al., 2010b), the UV filter 4-

methylbenzylidene camphor (4-MBC) (Badia-Fabregat et al., 2012) and several agrochemicals (Mir-Tutusaus et al., 2014).

Fungal cytochrome P450, shares some similarities with its mammalian and human counterparts (Stojan et al., 2014). These similarities include the capacity of forming glucuronides and conjugates in general (Bezalel et al., 1996). In humans, conjugation increases water solubility of xenobiotics so they can be excreted via urine (Dalgaard and Larsen, 1999; Lynn et al., 1978). WRF, however, have been consistently reported to reverse such modifications and deconjugate human conjugates (Badia-Fabregat et al., 2015a; Mir-Tutusaus et al., 2017).

2.2. Advantages and disadvantages of WRF systems vs. bacterial treatment

The fungal enzymatic systems are an important capability that supports WRF's suitability for bioremediation of micropollutants from wastewater, but it is not the only one –and they come with some disadvantages too.

Micropollutants are typically found in wastewater streams at trace concentrations. This fact poses a difficulty for bacterial degradation as bacteria typically use the contaminants as growth substrates. If the pollutant is present at a low concentration, the bacterial species that is supposedly able to degrade it will not be able to colonize the matrix (Harms et al., 2011). Degradation of organic pollutants in white-rot fungi, on the other hand, is part of the secondary metabolism. In other words, fungi need a carbon source other than the contaminant to grow, meaning that WRF transform micropollutants co-metabolically (Wen et al., 2011). This does not mean that WRF cannot metabolize the micropollutant: *T. versicolor* could metabolize, mineralize and integrate some micropollutants such as diclofenac and benzophenone-3 into the fungus'

amino acids (Badia-Fabregat et al., 2014; Marco-Urrea et al., 2010b, 2010c). However, the concentration of micropollutants is insufficient to maintain fungal growth and a secondary carbon source is therefore needed. On one hand, this feature enables WRF to attack the micropollutants present in the wastewater even at low concentrations. On the other hand, the need for an additional carbon source constitutes a drawback over bacterial treatment.

Municipal and municipal-like wastewater commonly contains a mixture of a wide range of trace organic pollutants: from caffeine and insect repellents such as N,N-diethyl-meta-toluamide (DEET) to sunscreens, preservatives, antibiotics, hormones and other pharmaceutically active compounds (Wang et al., 2014; Yang et al., 2017). It is noteworthy that although they are found at trace concentrations, they retain high biological activities. Bacteria are usually less versatile when treating combinations of pollutants: a specific bacterial species can be a good degrader of a single or a small subset of similar micropollutants and this constitutes an advantage when treating a waste stream contaminated with a single micropollutant. But bacteria in general have difficulties when removing mixtures of contaminants. Conventional activated sludge, for instance, does no degrade most of pharmaceuticals and personal care products in municipal wastewater (Verlicchi et al., 2015, 2012). Recently an interesting review has been published about the organic micropollutants removal in conventional biological wastewater treatment where the requirement of hybrid treatment is pointed out, including the use of WRF (Grandclément et al., 2017). Authors suggest the need of studying the influence of the operational conditions, which is one of the objectives of this review. White-rot fungi's non-specific enzymatic machinery, on the other hand, is especially well suited for coping with this scenario, as their ability to degrade mixtures of several contaminants has been widely

demonstrated (Mir-Tutusaus et al., 2014; Shreve et al., 2016; Valentín et al., 2007). However, the pH of municipal and municipal-like wastewater is commonly around 7, while an effective fungal treatment usually requires pH 4.5. This drawback could be easily solved at expense of increasing the process cost.

In regards to the interaction between fungal and bacterial species, studies about the evolution of the microbial communities are scarce. However it has been found that bacteria and fungi can show a positive synergistic effect. This was hypothesized between fungal and bacterial enzymes that led to an increase removal percentage of several pollutants in non-sterile wastewater treatment in contrast to sterile treatment (Gros et al., 2014). This is regarded as a key aspect that requires further research in the future.

Additionally, although fungal systems have been regarded as a cost-effective solution for micropollutant removal, it is important to note that the application cost strongly depends on several factors: cost for inoculum and biomass production, requirement for operating conditions adjustment (e.g., pH adjustment), need for adding unit processes and hydraulic retention time among others.

Finally, some waste effluents cannot be treated with fungi: WRF systems are not good candidates for anoxic groundwater bioremediation, for example, where oxygen is scarce. WRF indeed need aerobic conditions for survival and activity whilst some bacterial species thrive in such environments and some can effectively degrade pollutants –e.g., dichloromethane fermentation in anaerobic conditions (Trueba-Santiso et al., 2017). However, aerobic waste effluents with concentrated pollutants pose a problem for conventional wastewater treatment processes: pulp and paper bleach industry effluent contains chlorinated and phenolic compounds;

olive oil mill effluent is acidic and contains toxic phenols; textile and dyestuff industry effluents contain structurally distinct dyes; pharmaceutical industry effluent might contain residues of the active compound produced (Harms et al., 2011). White-rot fungal processes, on the other hand, have been reported to survive these conditions and degrade the pollutants in such waste streams (Nogueira et al., 2015; Ntougias et al., 2015; Van Driessel and Christov, 2001; Zhuo et al., 2011). Therefore, white-rot fungal based treatments can be regarded as a good option for on-site treatment of these wastewaters.

3. White-rot fungi and continuous wastewater treatment for micropollutants removal

In this section several continuous fungal operations treating a variety of micropollutants, summarized in Tables 1-3, are reviewed. Special interest is invested in works carried out using whole-cell cultures in non-sterile conditions because they portrait a more realistic picture of the technology.

3.1. Pharmaceutically active compounds

Pharmaceutically active compounds, or PhACs, are molecules that enter the environment and remain active, either as unmetabolized parent compounds or as pharmaceutically active metabolites (also referred as transformation products, or TPs). Drugs are administered to humans or animals and reach the environment via excretory systems in an unmodified, partially metabolized or completely metabolized state (Ebele et al., 2017). These molecules can promote drug tolerance or resistance to the original target organisms (e.g. antibiotic resistance in bacteria, or analgesic tolerance in humans) and unwanted effects in non-target organisms (e.g. alteration of sex ratio and decreased fertility) (Annamalai and Namasivayam, 2015; Jorgensen and Halling-Sorensen, 2000) even at a very low concentration. The intended biological activity

allowed scientists to categorize several compounds into families: analgesics and anti-inflammatories, antibiotics, psychiatric drugs, beta-blockers or lipid regulators, among many others. In this section continuous PhAC removal is reviewed (and summarized in Table 1), opening with sterile and defined matrices and moving on to non-sterile and complex matrices such as wastewater.

Although several fungal species have been found to have PhAC degradation capabilities and showed promising results (Castellet-Rovira et al., 2018), continuous bioreactor treatments focused mainly on *Trametes versicolor* and *Phanerochaete chrysosporium*. *P. chrysosporium* was investigated in several operation modes and reactor configurations for the continuous removal of analgesics and anti-inflammatories diclofenac (DCF), ibuprofen (IBU) and naproxen (NPX), and psychiatric drugs carbamazepine (CBZ) and diazepam in sterile defined media. Nearly complete removal of DCF, IBU and NPX was achieved when biomass was auto-immobilized in the form of pellets and stirred tanks were used with a hydraulic retention time (HRT) of 1 d (Rodarte-morales et al., 2012; Rodarte-Morales et al., 2011). The fungus was not able to remove diazepam and an unstable CBZ removal of 0-63% was achieved when spiking at 0.5 $mg \cdot L^{-1}$. Similar results were achieved when operating a fixed bed reactor, even in a 100-day long operation: complete removal of analgesics and anti-inflammatories and limited and unstable removal of diazepam (0-30%) and CBZ (0-40%) (Rodarte-Morales et al., 2012). These series of studies exemplified a general trend in fungal PhAC degradation: analgesics and anti-inflammatories are usually well removed whilst the psychiatric drugs family is more recalcitrant. The possibility of CBZ and the sulfonamide antibiotics sulfamethazine (SMT), sulfathiazole (STZ) and sulfapyridine (SPY) removal by *T. versicolor* pellets was investigated in a sterile

fluidized bed bioreactor treating defined media. Jelic et al. (2012) and Rodríguez-rodríguez et al. (2012) obtained a 54% removal of CBZ when spiking with 200 $\mu g \cdot L^{-1}$ and >94% removal of the sulfonamides spiked at 5 $mg \cdot L^{-1}$.

Some studies used non-sterile defined media, sometimes referred as non-sterile synthetic wastewater, as an approach to real application. Nguyen et al. (2013) and Yang et al. (2013a) used this approach to study the behavior of a membrane bioreactor (MBR) inoculated with *T. versicolor* lumps with an HRT of 2 d. Again, analgesics and anti-inflammatories were highly removed (salicylic acid, ketoprofen, ibuprofen, naproxen), with the exception of diclofenac, with an unstable removal of 0-60%. CBZ and the antibiotic metronidazole were poorly removed at 21 and 38% removal, respectively. Psychiatric drugs amitriptyline and primidone were also well removed. Long-term operations of 165 d and 160 d were achieved by Li et al. (2016, 2015b) using immobilized *P. chrysosporium* in a countercurrent seepage bioreactor and a rotating suspension cartridge reactor treating naproxen and carbamazepine spiked non-sterile defined media. The operations removed up to 70-90% of carbamazepine, value not achieved in any other study reviewed. A similar non-sterile media was compared with the use of non-sterile spiked municipal wastewater in a plate bioreactor described in Zhang and Geißen (2012). Immobilized *P. chrysosporium* removed in that operation an 80 and 60% of CBZ in the defined media and wastewater, respectively.

In order to shed light on the effect of sterility, Gros et al. (2014) operated the same 10 L fluidized bed reactor with sterile and non-sterile hospital wastewater (two wastewaters were collected on different days) inoculated with *T. versicolor*. The X-ray contrast agent iopromide and the antibiotic ofloxacin were removed up to 87 and 98.5%, respectively, in the sterile reactor

and 65.4 and 99%, respectively, in the non-sterile reactor. Further approaching real-life application, several studies were carried out using real wastewater. Badia-Fabregat et al. (2015b) operated a fluidized bed reactor with *T. versicolor* pellets treating non-spiked, non-sterile veterinary hospital wastewater. Some compounds in the analgesics and anti-inflammatory family were well removed, but some exhibited an increase in their concentration (ketoprofen, piroxicam, diclofenac, indomethacine). An impressive 83% removal was obtained for diazepam and complete removal of ranitidine, clopidrogel and the antibiotic ciprofloxacin were achieved, but other pharmaceuticals were poorly removed. In a hospital wastewater spiked with ketoprofen and ibuprofen, 80 and 100% removal values were achieved using a similar fungal system (Mir-Tutusaus et al., 2016). Comparing both studies, it is interesting to note that ketoprofen was well removed in the spiked matrix, but its concentration rose when the matrix was not spiked. This was related to conjugation/deconjugation processes, which are briefly discussed in sections 2.1 and 5. A similar non-spiked study used non-sterile hospital wastewater pretreated with a coagulation-flocculation treatment to feed a similar fluidized bed bioreactor inoculated with *T. versicolor* pellets (Mir-Tutusaus et al., 2017). The reactor was operated for 56 d and nearly complete removal was achieved for the analgesics and anti-inflammatories family with the exception of ketoprofen, whose concentration rose, which was in accordance to Badia-Fabregat et al. (2015b). Around 60% of antibiotics were removed and psychiatric drugs were well removed overall.

3.2. Endocrine disruptors

Previous studies have confirmed significant removal of various trace organic contaminants by white-rot fungal cultures under sterile batch test conditions. However, little is known about

endocrine disruptor compounds' removal in fungal reactors operating in continuous mode; such studies are summarized in Table 2. *Trametes versicolor* was the most investigated white-rot fungus for the removal of these contaminants. Among the various types of pollutants, endocrine disruptors are receiving increasing attention as they are widespread and can pose serious risks to the environment and public health, even at low concentrations (Auriol et al., 2006). Indeed, these chemicals interfere with the hormone systems and produce adverse developmental, reproductive, neurological, and immunological effects in mammals. These compounds can be found in many products including plastic bottles, metal food cans, detergents, flame retardants, food, toys, cosmetics, and pesticides (Yang et al., 2017).

Estrogen compounds including natural ones, estrone (E1), 17β-estradiol (E2), estriol (E3), and synthetic 17α-ethinylestradiol (EE2) are commonly detected in sewage effluents and considered to be significant contributors to the estrogenic activity of wastewaters due to their high endocrine disruptor activity even at extremely low concentrations (Cabana et al., 2007; Shreve et al., 2016). Removal of these compounds in continuous mode using white-rot-fungi has been reported by some authors. Blánquez and Guieysse (2008) explored the potential of the white-rot fungus *Trametes versicolor* to biodegrade E2 and EE2 in a fluidized bed bioreactor operated during 26 days at a hydraulic retention time of 120 h. The results showed that E2 and EE2 were completely removed at volumetric removal rates of 0.16 and 0.09 mg l^{-1} h^{-1}, respectively, when fed at 18.8 and 7.3 mg l^{-1}, respectively. Shreve et al. (2016) explored the potential of the same fungus *T. versicolor* using the strain NRRL 66313 to continuously remove E1, E2 and EE2 from a mixture of nine trace organic contaminants with 350 μg·L^{-1} concentration each and during 8 days. The results showed that *T. versicolor* was able to decrease the estrogenic activity of the mixture and

especially of the target contaminants (more than 71%) with the following trend E2 > E1 > EE2. Nguyen et al. (2013) studied the continuous removal of 30 trace organic contaminants, E1, E2 EE2, E3 and 17-b-estradiol-17-acetate among them, in a fungus-augmented bioreactor. The reactor contained the white-rot fungus *T. versicolor* and activated sludge and was operated for 110 d. It was fed continuously with synthetic wastewater spiked with the selected contaminants each with a concentration of approximately 5 $\mu g \cdot L^{-1}$. Data from this study highlighted the high removal of these compounds (> 90%) by the fungus-augmented bioreactor. The degradation of the same endocrine disrupting compounds, except 17-b-estradiol-17-acetate, was also recently investigated by Křesinová et al. (2017) using *Pleurotus ostreatus* HK 35. The strain was first, tested in a laboratory-scale continuous-flow reactor and then in a pilot bioreactor under non-sterile conditions. Results revealed that the EDC degradation in the trickle-bed bioreactor containing the mixed culture of the fungus and wastewater-autochthonous bacteria was very efficient in both cases. In the same work, the authors investigated also the bioreactor inoculated with the same strain as a tertiary treatment step to remove EDC, including E1 and EE2, from effluent of secondary treatment. Results also showed the potential of *P. ostreatus* HK 35 to remove these compounds and that 100 and 71% of E1 and EE2 were removed, respectively, within 24 hours.

Phenolic compounds, mainly bisphenol A (2,2-bis (4-hydroxyphenol) propane), nonylphenol (4-nonylphenol), and triclosan (5-chloro-2(2,4-dichloro-phenoxy)phenol) are xenobiotic compounds frequently detected in receiving waters downstream of areas of intense urbanization (Boyd et al., 2003; Kolpin et al., 2002). These chemicals are classified as endocrine disruptors since they can mimic or interfere with the hormonal system of different organisms (Cabana et al.,

2009; Naylor, 1995). Although they have many orders of magnitude lower estrogenic activity, their elevated concentrations in wastewater drew attention to these EDC. Bisphenol A is used as raw material for the production of polycarbonates and epoxy resins; nonylphenol mainly originates from the degradation of nonylphenol polyethoxylates, a widely used industrial surfactant and triclosan is widely used in soaps, mouthwashes, toothpastes and other products in household personal care and hospital applications. The application of white-rot fungi in continuous mode for the treatment of these phenolic compounds has been scarcely described. Continuous removal of Bisphenol A was studied by Yang et al. (2013) in a membrane bioreactor (MBR) inoculated with *T. versicolor* and operated in non-sterile conditions for three months. Results showed that the performance of the fungal MBR was dependent on trace organic contaminants loading. Indeed, 80 to 90% were removed at an HRT of two days and bisphenol A loading of 475 $mg \cdot L^{-1} d^{-1}$. Continuous removal of Bisphenol A was also reported in other studies and reached 75% in a fungus-augmented bioreactor operated during 110 d (Nguyen et al., 2013) and 61.9 % in the conditions of the study described previously by Shreve et al. (2016).

Regarding the antibacterial agent triclosan, it has been reported to be well removed (>95%) in continuous mode using *T. versicolor* at an initial concentration of 5 $\mu g \cdot L^{-1}$ in synthetic medium (Nguyen et al., 2013). However, low (34%) or no removal was observed using the strains *T. versicolor* NRRL 66313 and *P. ostreatus* HK 35 at initial concentrations of 25 $ng \cdot L^{-1}$ and 350 $\mu g \cdot L^{-1}$ respectively, in an effluent from secondary treatment (Kresinová et al., 2017; Shreve et al., 2016). Nguyen et al. (2013) also reported the removal of benzophenone, octocrylene and oxybenzone (three UV filters) with values of 68, 90 and 96%, respectively. However, Shreve et al. (2016) observed no removal of oxybenzone.

3.3. Pesticides

Few studies have investigated pesticide removal in continuous mode using different white-rot fungi and conditions, and they are summarized in Table 3. The potential of the white-rot fungus *Bjerkandera adusta* for the degradation of the insecticide hexachlorocyclohexane (HCH) in a spiked soil in a slurry system was investigated by Quintero et al. (2007). Bioremediation studies in the reactor were performed for 30 d and the operational conditions tested were the solid load (10% and 30%) and concentration of the pollutants in the soil (25 and 100 $mg \cdot kg^{-1}$). The results showed that higher degradation percentages were obtained for a solid concentration of 10% and a concentration for each isomer of 25 $mg \cdot kg^{-1}$ and were of 94.5%, 94.5%, 78.5% and 66.1%, for α-, γ-, δ- and β-HCH isomers, respectively.

The performance of a continuous packed bed bioreactor degrading the organophosphorus insecticide chlorpyrifos by the fungus *Aspergillus* sp. was studied at varying insecticide loading rates by Yadav et al. (2015). *Aspergillus* sp. is not a white-rot fungus but it was found to be quite efficient in the biodegradation of chlorpyrifos and its removal efficiency varied from 68 to 89% with the flow rate ranging from 10 to 40 $mL \cdot h^{-1}$ and the HRT from 24 to 100 h. Results also showed that the continuous packed bed bioreactor was able to regains its performance quickly after the perturbation in the flow rate. The potential of the same fungus *Aspergillus niger* to degrade continuously an herbicide, atrazine, in wastewater was evaluated by Marinho et al. (2017).

T. versicolor showed potential in the biodegradation of clofibric acid in a fluidized bed bioreactor. The study operated for 24 d a continuous reactor with an HRT of 4 days and achieved a 80% removal (Cruz-Morató et al., 2013b). Interestingly, the identification of transformation

products and a toxicity assessment showed that the treated effluent was more toxic than the initial feed, probably due to the presence of hydroxyl-clofibric acid. Continuous removal of six pesticides, namely, atrazine, propoxur, fenoprop, ametryn, clofibric acid and pentachlorophenol, was investigated by Nguyen et al. (2013) with the same fungus in a MBR treating synthetic medium. The main results showed that fungus-augmented reactor achieved good removal of fenoprop (57%), clofibric acid (65%) and pentachlorophenol (92%) compared to conventional MBR. Toxicity assays were not performed in this case. The effect of a continuous dosing of a mediator (1-hydroxy benzotriazole, HBT) to the fungus-augmented MBR was also investigated during the last 30 days of operation. The results showed no significant difference in removal of atrazine and ametryn by the MBR, even after doubling the mediator dose to 10 μM. Shreve et al. (2016) observed no removal of atrazine and N,N-diethyl-3-methylbenzamide (DEET) within a mixture of nine contaminants spiked on sterile WWTP effluent.

3.4. Industrial chemicals

Continuous treatment of industrial chemicals has been also examined only by few researchers and the studies are summarized in Table 3. Palli et al. (2016) investigated the biodegradation of 2-naphthalensulfonic acid polymers (NSAP) in a wastewater in a continuous packed bed bioreactor working for three months under non-sterile conditions. The bioreactors were inoculated by *B. adusta* and *P. ostreatus* immobilized on straw. The results showed that the fungus *B. adusta* exhibited a limited enzymatic activity and was not able to remove the tested

contaminant. However, the reactor inoculated with *P. ostreatus* showed a stable laccase activity during the whole experiment and noticeable NSAP biodegradation was achieved after two weeks of work and remained until the end of the experiment (30 to 60 %). In another study, high removal (> 95%) of two industrial chemicals, namely 4-tert-Butylphenol and 4-tert-Octylphenol, among thirty contaminants, was observed in an augmented fungal MBR (Nguyen et al., 2013).

4. Limitations of fungal based systems and how to overcome them

Despite all the potentialities of WRF, and the high amount of interesting studies about fungi being used for micropollutant removal, fungal systems for wastewater treatment are not being commonly applied at industrial scale. In this section, we review the main drawbacks of the technology and how can they be overcome.

4.1. Need for nutrient addition

As discussed in section 2.2, although organic micropollutants contain carbon, some WRF need an additional assimilable carbon source for growth and survival. Wastewater usually contains organic carbon and nitrogen (Verlicchi et al., 2010), both needed for microbial growth, and bacteria are perfectly capable of assimilating both. In the case of WRF, most experiments used glucose-based or malt extract-based spiked media (a.k.a. synthetic wastewater) and few studies can be found using real wastewater. The need for nutrient addition in real wastewater treatments by WRF was identified only after using real wastewater. Cruz-Morató et al. (2013) and Badia-Fabregat et al. (2015a) highlighted the need of glucose and ammonium tartrate addition for maintaining pelleted *T. versicolor* biological activity and enzymatic production in a fluidized bed bioreactor treating wastewater. Zhang and Geißen (2012) also found that glucose and ammonium tartrate addition were required for carbamazepine removal in a plate bioreactor inoculated with

polyether foam-immobilized *P. chrysosporium* treating WWTP effluent. Other studies using fluidized bed bioreactors and treating flocculated hospital wastewater obtained similar results when adding ammonium chloride instead of ammonium tartrate (Mir-Tutusaus et al., 2017, 2016). In the reviewed literature, common nutrient addition rates ranged between 343 – 1453 mg·g dry cell weight $(DCW)^{-1}\cdot d^{-1}$ of glucose and 0.77 - 1.98 mg·g $DCW^{-1}\cdot d^{-1}$ of ammonium tartrate. However, some WRF were able to assimilate organic components (measured as chemical oxygen demand, COD) from wastewater: Palli et al. (2017) operated a fluidized bed reactor with *Pleurotus ostreatus* and observed significant growth of the fungus and reduction in the COD concentration. In those cases where a fungal species able to assimilate wastewater COD is used, there is no need for nutrient addition. This in turn could reduce bacterial growth, but overgrown fungal biomass should then be purged regularly. Nevertheless, it can be fairly accepted that nutrient addition can be needed to operate a white-rot fungal reactor for the treatment of wastewater. This poses a problem to full scale application, as the cost of glucose and nitrogen addition would be high, especially taking into account the large volumes of wastewater treated in WWTPs, and potentially increase the COD and nitrogen load.

This limitation could be partially overcome (i) by optimizing the nutrient addition, because when nutrients are added at consumption rate lower quantities are needed and nutrients' concentration in the effluent remains very low, therefore not increasing COD or nitrogen load. This in turn prevents overgrowth of fungal biomass; (ii) by replacing the glucose and ammonium tartrate/chloride by cheaper products; or (iii) by reimagining the use of the technology: white-rot fungal systems could be viable, even taking into account the costs of nutrient addition, when smaller volumes of micropollutant-contaminated wastewater are treated. This is the case, for

example, of hospital wastewater, veterinary hospital wastewater and several industrial wastewaters (Verlicchi et al., 2010). A fourth answer to this limitation is (iv) the immobilization of fungal biomass onto lignocellulosic materials. These substrates act also as carbon and nitrogen sources for WRF, thus avoiding the need of nutrient addition (Ehlers and Rose, 2005; Lu et al., 2009; Torán et al., 2017). It is worth noting that the use of a lignocellulosic material may lead to the release of recalcitrant compounds, e.g. tannins or phenolic compounds (Ramos et al., 2013). However, an advantage is that lignocellulosic materials are very abundant and are usually byproducts of other industries, reducing their price (Dashtban et al., 2010; Leonowicz et al., 1999).

4.2. Immobilization of fungal biomass

Fungal dispersed mycelium usually causes bioreactor operation difficulties such as growth on the reactor walls and agitators, foaming and increased need of mixing and oxygen supply. The immobilization of fungal biomass overcomes most of these difficulties –or reduces them.

The immobilization can be accomplished by the growth of the fungus in form of pellets (auto-immobilization). Fungal pellets are spherical aggregates of interweaved hyphae with a size usually in the range of several hundred micrometers to several millimeters (Espinosa-Ortiz et al., 2015). This immobilization is usually accomplished by growing the fungus in Erlenmeyers with liquid media under shaking conditions. Quite a few studies have dealt with the pelletization of different fungal species, the optimal pellet diameter and the study of mass and oxygen transfer into the pelleted biomass (Borràs et al., 2008; Casas López et al., 2005; Feng et al., 2004; Leštan and Lamar, 1999; Sharma and Padwal-Desai, 1985; Sitanggang et al., 2010; Wittier et al., 1986). Some studies have reported successful pellet production in a fluidized bed reactor, even in a

pilot-scale bioreactor, thus enabling the upscaling of the technology (Borràs et al., 2008; Mir-Tutusaus et al., 2017). Additionally, Espinosa-Ortiz et al. (2015) reviewed several fungal pelleted reactor configurations with the perspective of treating wastewater.

The immobilization can also be carried out by growing the fungus onto a carrier. Some studies have done so using inert carriers such as polyurethane foam cubes (Li et al., 2016; Yadav et al., 2015). Gao et al. (2008) listed amongst the advantages of immobilizing *P. chrysosporium* in polyurethane foam the improved survival and increased enzymatic activity of the fungus in non-sterile cultures. But taking into account WRF's ability of degrading lignin, cellulose and hemicellulose, several other authors have looked into the immobilization onto non-inert carriers such as wood chips, serving both as support and carbon source (Li et al., 2015; Pedroza-Rodríguez and Rodríguez-Vázquez, 2013; Rodarte-Morales et al., 2012; Sirtori et al., 2009). Interestingly, when Ehlers and Rose (2005) immobilized several WRF in pine chips, fungi were shown to penetrate the wood, possibly using the cellulose and hemicelluloses as carbon source. In this case, WRF not only benefited from the immobilization, but bacteria were not able to use the carbon source, hence avoiding substrate competition. Recent studies have also reported improved micropollutant degradation and fungal survival with *T. versicolor* immobilized in wood chips, even when treating real wastewater (Torán et al., 2017).

In general, immobilization and auto-immobilization leaded to more robust operations in non-sterile conditions (Hai et al., 2013; Leidig et al., 1999; Nilsson et al., 2006; Tang et al., 2011). Experiments with immobilized biomass tend to use fixed-bed column reactors (their low shear stress helps the adhesion of the biomass on the support) rather than the stirred-tank or fluidized bed reactors usually used with pelleted biomass.

4.3. Competition with autochthonous microorganisms

The decline in micropollutant removal observed in several studies has been largely attributed to bacterial contamination and it has been identified as the main bottleneck of the technology (Espinosa-Ortiz et al., 2015; Gao et al., 2008; Hai et al., 2013, 2008; Libra et al., 2003). Indeed, bacteria has been shown to exert competitive pressure for the substrate, thus leading to the loss of fungal biomass, and to destabilize fungal enzymes (Hai et al., 2008; Libra et al., 2003). For that reason, researchers have since proposed a wide range of alternatives for dealing with this limitation.

4.3.1. Favoring fungal growth

Favoring fungal growth usually involves supplying the conditions that distinctively favor WRF over bacteria. These strategies include operation at optimal fungal pH, immobilization of fungal biomass, periodical biomass renewal and optimizing the carbon-to-nitrogen ratio (C/N ratio) of the nutrients supplied.

- **Operation at optimal fungal pH**. Most white-rot fungi's optimal pH is acidic; not surprisingly, lignin modifying enzymes' optimal pH is also acidic (Pazarlioglu et al., 2005). Although a specific bacterial species might find it difficult to grow at acidic pH, bacteria is a diverse domain and acidic pH does not suppress bacterial growth. However, pH too acidic ceased enzyme production of *T. versicolor* and led to the loss of pelleted morphology in a fluidized-bed reactor (Borràs et al., 2008; Libra et al., 2003). Therefore, acidic pH does not *distinctively* favor fungi over bacteria, but it does improve the viability and activity of WRF.

- **Partial biomass renovation**. When growth-limiting culture conditions are implemented the biomass concentration is nearly constant because the low nutrient addition is used mainly for biomass maintenance. In this case the biomass retained in the bioreactor ages over time. In this scenario, partial biomass renovation was developed as a strategy for stabilizing the age of fungal biomass in a sterile treatment, thus extending the operational time (Blánquez et al., 2006). They purged 1/3 of the fungal biomass in the reactor and added the same amount of fresh biomass every week, obtaining a solids/cells retention time of 21 d. The work concluded that partial biomass renovation helped in maintaining a biomass age distribution constant as well as their activity. So a pseudo steady state was obtained for the fungus in the bioreactor. The strategy was continued in several sterile operations (Blánquez and Guieysse, 2008) and in a non-sterile treatment of wastewater by Badia-Fabregat et al. (2015b) in an attempt to improve the enzymatic production and integrity of pellets. It was also successfully applied in a non-sterile operation of wastewater pretreated with a coagulation-flocculation process, allowing for a 56-day treatment (Mir-Tutusaus et al., 2017). In summary, the substitution of old biomass by fresh one allowed for a more stable fungal population in the reactors, in turn maintaining enzymatic activity for a longer period of time and favoring white-rot fungal colonization.
- **Carbon-to-nitrogen ratio**. In systems where nutrient addition is needed –i.e. where biomass is not immobilized in lignocellulosic substrates and/or the fungus is not able to assimilate nutrients present in the wastewater–, the ratio between carbon and nitrogen may play a role in favoring fungal over bacterial populations. On the one hand, high C/N ratios mimic ligninolytic conditions (wood has a high C/N ratio), increasing white-rot fungal

production of lignin modifying enzymes (Eggert et al., 1996); and not contrarily, limiting conditions of carbon or nitrogen have also been reported to enhance LME production (Viswanath et al., 2014). On the other hand, lower carbon-to-nitrogen ratios favor fungal growth over bacterial growth (Demoling et al., 2007; Rousk and Bååth, 2007). It is important to notice that lower ratios do not favor white-rot fungi exclusively, but rather the growth of fungal species in general. Therefore, a compromise must be found between favoring fungal growth over bacteria and favoring LME production. However, one should take into account that LME production has rarely been linked to an increase of micropollutant removal. For example, in a recent publication treating flocculated wastewater in a fluidized bed bioreactor, a rather low C/N ratio of 7.5 was chosen in terms of PhAC degradation and biomass integrity (Mir-Tutusaus et al., in press).

- **Immobilization.** In addition to the advantages of immobilization discussed in section 4.2, auto-immobilization of WRF in the form of pellets allows a high concentration of fungus inside the reactor, thus hindering bacterial colonization. If the immobilization is carried out on lignocellulosic carriers the fungal concentration tends to be lower, but most bacterial species find it difficult to grow on lignocellulosic substrates.

4.3.2. Washing out bacteria

Another strategy for overcoming the competition with native microorganisms is by means of decoupling the hydraulic retention time (HRT) and solids retention time (SRT), sometimes also referred as cellular retention time (CRT). The purpose of these strategies is to keep the fungal biomass in the reactor while washing out the bacteria and other microorganisms, therefore

increasing the retention time of WRF while keeping an HRT able to wash out the other microorganisms.

In order to achieve this decoupling, some authors auto-immobilized the WRF, typically in the form of pellets while others immobilized the fungi on inert carriers or lignocellulosic substrates, as discussed in section 4.2. A third option for decoupling HRT and fungal retention time is by the use of membrane technology. Membranes are widely used and can be found at industrial scale in several WWTPs (Joss et al., 2006; Rubirola et al., 2014). They allow for higher SRT and have been successfully applied with fungal biomass for the removal of organic micropollutants at laboratory scale (Hai et al., 2009; Nguyen et al., 2013; Yang et al., 2013).Van Leeuwen et al. (2003) described a technology using 100 μm microscreens that allowed for production of the fungus *Rhizopus* (not a white-rot fungus) under non-aseptic conditions thanks to the manipulation of HRT and SRT.

These four approaches allow for the retention of fungal biomass inside the reactor, therefore permitting the decrease of the HRT without affecting the SRT. A lower HRT leads to the washout of non-attached microorganisms, and bacterial concentration has been linked with the loss of degradation capacity, enzymatic production and viability of WRF (Blánquez et al., 2008; Hai et al., 2013, 2009; Mir-Tutusaus et al., 2016). Therefore, lower HRT favor white-rot fungal viability by washing out non-attached microorganisms. However, it is noteworthy that bacteria can attach to virtually everything, including pellets, immobilized fungal biomass, inert carriers and reactor surface (Fletcher, 1994). While fungal survival might be improved, lower HRTs often meant lower degradation of several contaminants by WRF: for example, Blánquez et al. (2007) reported reduced decolorization of a textile dye when lower HRTs were applied, similarly

to Asses et al. (2009). Moreover, washing out of bacteria comes inevitably with the washout of extracellular enzymes and mediators produced by the fungus (Badia-Fabregat et al., 2017; Nguyen et al., 2013). However, as reviewed in sections 2 and 3, not only extracellular enzymes play a role in microcontaminant degradation. In fact, several authors reported concentration of LMEs not being crucial to maintain good removal percentages (Anastasi et al., 2010; Blánquez et al., 2004; Yang et al., 2013). In spite of that, maintaining a sufficient concentration of LMEs in the reactor is desirable for compounds whose biotransformation is LME-dependent.

In summary, both HRT and SRT must be optimized in order to achieve a compromise between bacteria-and-enzyme washout, micropollutant removal and fungal survival.

4.3.3. Suppressing bacteria

Another strategy for assisting fungi in the competition with autochthonous microorganisms is the direct suppression of bacteria. This could obviously be achieved by sterilization, but it is not feasible in the wastewater treatment industry. Regardless, two approaches have been studied in order to reduce the bacterial count.

Sankaran et al. (2008) suggested the use of ozone (O_3) as a selective disinfectant in order to decrease bacterial contamination in a non-sterile continuous fungal cultivation on corn-processing wastewater. The aim of the work was the production of fungal biomass, rather than COD or micropollutant removal; that is why the researchers used very high dosages of ozone (57 $mg \cdot L^{-1}$), while ozone doses in full scale WWTPs range between 5 and 15 $mg \cdot L^{-1}$ (Ternes et al., 2003; Verlicchi et al., 2010). Ozonation behaves similarly to acidic pH in the sense that it favors most fungal species over bacteria. In fact, Sankaran et al. (2008) inoculated the reactor with *R. oligosporus* but the fungal population was replaced by a wastewater-native fungus. Cheng et al. (2013) used

ozone as a bactericide in a white-rot fungal dye-decolorization continuous operation, thus maintaining the bacterial concentration at around 10^5 CFU·mL^{-1}. The study reported a 99.4% inhibition of contaminating bacteria and the involvement of ozone in the degradation of the Acid Blue 45 dye. Indeed, ozone has been reported to improve biodegradability of refractory organic matter and to degrade several micropollutants (Contreras et al., 2003; Fujioka et al., 2014; Gomes et al., 2017; Kusvuran and Yildirim, 2013; Ternes et al., 2003; Yang et al., 2016). Therefore, care must be taken when using ozonation as a disinfectant in assigning removal efficiency to the WRF and to the ozonation itself.

The addition of pretreatments can potentially reduce the inlet concentration of bacteria. A recent study successfully extended the operation of a *T. versicolor* fluidized bed reactor treating hospital wastewater from 10 to 28 days (Mir-Tutusaus et al., 2016). Specifically, a coagulation-flocculation pretreatment reduced the bacterial count of the influent wastewater from 10^7-10^8 to 10^3-10^5 CFU·mL^{-1}, allowing for a longer-term operation. Coagulation and flocculation processes have been largely applied in WWTPs and are regarded as cost-effective (Liu et al., 2012; López-Maldonado et al., 2014). Therefore, the addition of this and other pretreatments might be a noteworthy strategy that enables WRF to operate with urban-like wastewaters.

4.3.4. A final note on non-sterility

Some studies in non-sterile conditions have been reviewed in this section. However, two groups can be distinguished: studies operating in non-sterile conditions with defined medium or tap water and studies using wastewater. The studies using defined medium or tap water usually rely on contamination by air-borne microorganisms and microorganisms present in non-sterile surfaces. Such contamination could be regarded as mild and operations tend to be longer. The

other group, using wastewater, deals with the contamination due to growth of native wastewater microorganisms. Bacterial count in those cases tends to be very high, the contamination could be regarded as heavy and the reactor operations tend to be shorter. The latter studies should be encouraged, because in addition to be a more reliable representation of real conditions, consortia formed in those operations could play a role in degradation of micropollutants and fungal metabolism intermediate products.

4.4. Fungal treatments require high HRTs

As discussed in section 4.3.2, low hydraulic retention times produced lower degradation for some micropollutants and higher loss of extracellular enzymes. Fungal treatments usually require a HRT of around 1-3 days for the removal of microcontaminants (Blánquez et al., 2008, 2007, Hai et al., 2009, 2008). In fact, micropollutant removal is usually improved by increasing HRT (when toxic compounds are not accumulated). Generally, WRF require higher HRTs to remove micropollutants than bacteria to remove organic matter. This adds a difficulty on combining a fungal treatment step on a conventional WWTP, reinforcing the idea of using white-rot fungal operations as on-site treatments in specific contaminated streams (enumerated in section 2.2 and 4.1). In those streams, the fungal process would be a treatment to decrease micropollutant concentration prior to discharge to the WWTP. If a fungal treatment were to be included in a conventional WWTP, some options could be considered: first, the increase of SRT or fungal concentration in the reactor could be optimized in order to allow higher removal efficiencies, thus enabling the coupling; second, low hydraulic retention times, between 6 to 12 h, are enough to remove several families of compounds such as analgesics, anti-inflammatories (Marco-Urrea et al., 2010a, 2009) and endocrine disruptors (Kresinová et al., 2017; Shreve et al., 2016),

although enzyme washout should be taken into consideration. Therefore, wastewaters containing mainly these families of pollutants could be treated with fungal systems at low HRTs.

5. Conclusions and future outlook

The fungal treatment of effluents containing organic micro-pollutants is a feasible alternative. However, the best strategy will depend on the wastewater to be treated, the final use of the treated wastewater and consequently the cost of the treatment.

In order to advance the technology towards industrial scale, sterility must be discarded. Wastewater sterilization is not feasible from an economic and environmental point of view, so fungal research in applied science should focus on non-sterile conditions. The difficulty of non-sterile fungal operations has been discussed, and it greatly shifts the focus on the research field: from establishing WRF's biodegradation capabilities to guaranteeing the fungus' survival and activity during the fungal operation. The biomass in the reactors are usually retained or immobilized. Therefore the biomass concentration in a continuous treatment, three different operation mode can be distinguished: (a) growth conditions due to high concentration of nutrients (either present in the wastewater or artificially supplied), where periodic purge is required to maintain the biomass level and good performance of the reactor; (b) growth limiting conditions with low nutrient supply, where biomass level remains constant but periodic partial biomass renovation is required to maintain the distribution of biomass age in the reactor and consequently maintaining the degradation capacity; and finally (c) biomass pre-grown on a ligninolytic material with no other nutrient addition, where the biomass concentration is lower than in the previous operation modes.

641 Besides favoring fungal survival over other microorganisms, some studies have focused in the
642 microbiological community evolution during fungal treatments. Such studies should be
643 encouraged, as bacterial and fungal interspecies interactions and its consequences in
644 micropollutant removal are not fully understood.

645 Similarly, the journey towards full scale operation requires the use of real, non-spiked
646 matrices. This should be no surprise, as the complexity of a real matrix –microbial diversity,
647 chemical composition, trace contaminants, etc. – is impossible to replicate in a defined medium.
648 In addition, the bacterial contamination problems when using real wastewater will be more
649 difficult to deal with, but they will be more similar to a real operation. Lastly, because real
650 matrices are a source of variability, successful fungal operations using real wastewater greatly
651 increase the systems' robustness.

652 The use of non-spiked real matrices, however, poses a big pressure on analytical techniques.
653 Not only are micropollutants found at a very low concentration, but they are also commonly
654 found in the form of glucuronides and other conjugated forms. Conjugated microcontaminants
655 are not usually detected by the current analytical techniques, thus undervaluing the concentration
656 of the pollutant studied. This in turn underestimates the removal capacity of WRF, as they have
657 been consistently described to deconjugate such compounds. Therefore, an effort should be made
658 to analyze all compounds in any form.

659 Reported experiences in pilot plant are still too scarce and consequently, the results obtained in
660 bench-scale reactors need to be validated at pilot plants before a full-scale application can be
661 considered.

Finally, the drawbacks of fungal wastewater treatment for the removal of recalcitrant organic micropollutants can be technologically overcome and the strategy will be established depending on the effluent quality required. WRF can be an alternative for the removal of organic micropollutants from real wastewater but further studies are necessary at pilot plant to full adapt the process to the real application.

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References

Anastasi, A., Spina, F., Prigione, V., Tigini, V., Giansanti, P., Varese, G.C., 2010. Scale-up of a bioprocess for textile wastewater treatment using Bjerkandera adusta. Bioresour. Technol. 101, 3067–3075. doi:10.1016/j.biortech.2009.12.067

Annamalai, J., Namasivayam, V., 2015. Endocrine disrupting chemicals in the atmosphere: Their effects on humans and wildlife. Environ. Int. 76, 78–97. doi:10.1016/j.envint.2014.12.006

Asses, N., Ayed, L., Bouallagui, H., Ben Rejeb, I., Gargouri, M., Hamdi, M., 2009. Use of Geotrichum candidum for olive mill wastewater treatment in submerged and static culture. Bioresour. Technol. 100, 2182–2188. doi:10.1016/j.biortech.2008.10.048

Auriol, M., Filali-Meknassi, Y., Tyagi, R.D., Adams, C.D., Surampalli, R.Y., 2006. Endocrine disrupting compounds removal from wastewater, a new challenge. Process Biochem. 41, 525–539. doi:10.1016/j.procbio.2005.09.017

Badia-Fabregat, M., Lucas, D., Gros, M., Rodríguez-Mozaz, S., Barceló, D., Caminal, G., Vicent, T., 2015a. Identification of some factors affecting pharmaceutical active compounds

(PhACs) removal in real wastewater. Case study of fungal treatment of reverse osmosis concentrate. J. Hazard. Mater. 283, 663–71. doi:10.1016/j.jhazmat.2014.10.007

Badia-Fabregat, M., Lucas, D., Pereira, M.A., Alves, M., Pennanen, T., Fritze, H., Rodríguez-Mozaz, S., Barceló, D., Vicent, T., Caminal, G., 2015b. Continuous fungal treatment of non-sterile veterinary hospital effluent: pharmaceuticals removal and microbial community assessment. Appl. Microbiol. Biotechnol. 100, 2401–2415. doi:10.1007/s00253-015-7105-0

Badia-Fabregat, M., Lucas, D., Tuomivirta, T., Fritze, H., Pennanen, T., Rodríguez-Mozaz, S., Barceló, D., Caminal, G., Vicent, T., 2017. Study of the effect of the bacterial and fungal communities present in real wastewater effluents on the performance of fungal treatments. Sci. Total Environ. 579, 366–377. doi:10.1016/j.scitotenv.2016.11.088

Badia-Fabregat, M., Rodríguez-Rodríguez, C.E., Gago-Ferrero, P., Olivares, A., Piña, B., Díaz-Cruz, M.S., Vicent, T., Barceló, D., Caminal, G., 2012. Degradation of UV filters in sewage sludge and 4-MBC in liquid medium by the ligninolytic fungus Trametes versicolor. J. Environ. Manage. 104, 114–120. doi:10.1016/j.jenvman.2012.03.039

Badia-Fabregat, M., Rosell, M., Caminal, G., Vicent, T., Marco-Urrea, E., 2014. Use of stable isotope probing to assess the fate of emerging contaminants degraded by white-rot fungus. Chemosphere 103, 336–342. doi:10.1016/j.chemosphere.2013.12.029

Bezalel, L.E.A., Hadar, Y., Fu, P.P., Freeman, J.P., Cerniglia, C.E., 1996. Metabolism of Phenanthrene by the White Rot Fungus Pleurotus ostreatus. Appl. Environ. Microbiol. 62, 2547–2553.

Blánquez, P., Caminal, G., Sarrà, M., Vicent, T., 2007. The effect of HRT on the decolourisation of the Grey Lanaset G textile dye by Trametes versicolor. Chem. Eng. J. 126, 163–169. doi:10.1016/j.cej.2006.09.007

Blánquez, P., Casas, N., Font, X., Gabarrell, X., Sarrà, M., Caminal, G., Vicent, T., 2004. Mechanism of textile metal dye biotransformation by Trametes versicolor. Water Res. 38, 2166–72. doi:10.1016/j.watres.2004.01.019

Blánquez, P., Guieysse, B., 2008. Continuous biodegradation of 17β-estradiol and 17α-ethynylestradiol by Trametes versicolor. J. Hazard. Mater. 150, 459–462. doi:10.1016/j.jhazmat.2007.09.085

Blánquez, P., Sarrà, M., Vicent, M.T., 2006. Study of the cellular retention time and the partial biomass renovation in a fungal decolourisation continuous process. Water Res. 40, 1650–6. doi:10.1016/j.watres.2006.02.010

Blánquez, P., Sarrà, M., Vicent, T., 2008. Development of a continuous process to adapt the textile wastewater treatment by fungi to industrial conditions. Process Biochem. 43, 1–7. doi:10.1016/j.procbio.2007.10.002

Bogan, B.W., Lamar, R.T., 1996. Polycyclic aromatic hydrocarbon-degrading capabilities of Phanerochaete laevis HHB-1625 and its extracellular ligninolytic enzymes. Appl. Environ.

Microbiol. 62, 1597–1603. doi:199662515971603

Borràs, E., Blánquez, P., Sarrà, M., Caminal, G., Vicent, T., 2008. Trametes versicolor pellets production: Low-cost medium and scale-up. Biochem. Eng. J. 42, 61–66. doi:10.1016/j.bej.2008.05.014

Boyd, G.R., Reemtsma, H., Grimm, D.A., Mitra, S., 2003. Pharmaceuticals and personal care products (PPCPs) in surface and treated waters of Louisiana, USA and Ontario, Canada. Sci. Total Environ. 311, 135–149. doi:10.1016/S0048-9697(03)00138-4

Cabana, H., Jiwan, J.-L.H., Rozenberg, R., Elisashvili, V., Penninckx, M., Agathos, S.N., Jones, J.P., 2007. 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 67, 770–778. doi:10.1016/j.chemosphere.2006.10.037

Cabana, H., Jones, J.P., Agathos, S.N., 2009. Utilization of cross-linked laccase aggregates in a perfusion basket reactor for the continuous elimination of endocrine-disrupting chemicals. Biotechnol. Bioeng. 102, 1582–1592. doi:10.1002/bit.22198

Camarero, S., Sarkar, S., Ruiz-Dueñas, F.J., Martínez, M.J., Martínez, Á.T., 1999. Description of a versatile peroxidase involved in the natural degradation of lignin that has both manganese peroxidase and lignin peroxidase substrate interaction sites. J. Biol. Chem. 274, 10324–10330. doi:10.1074/jbc.274.15.10324

Cañas, A.I., Camarero, S., 2010. Laccases and their natural mediators: Biotechnological tools for sustainable eco-friendly processes. Biotechnol. Adv. 28, 694–705. doi:10.1016/j.biotechadv.2010.05.002

Casas López, J.L., Sánchez Pérez, J.A., Fernández Sevilla, J.M., Rodríguez Porcel, E.M., Chisti, Y., 2005. Pellet morphology, culture rheology and lovastatin production in cultures of Aspergillus terreus. J. Biotechnol. 116, 61–77. doi:10.1016/j.jbiotec.2004.10.005

Castellet-Rovira, F., Lucas, D., Villagrasa, M., Rodríguez-Mozaz, S., Barceló, D., Sarrà, M., 2018. Stropharia rugosoannulata and Gymnopilus luteofolius?: Promising fungal species for pharmaceutical biodegradation in contaminated water. J. Environ. Manage. 207, 396–404. doi:10.1016/j.jenvman.2017.07.052

Cha, C.-J., Doerge, D.R., Cerniglia, C.E., 2001. Biotransformation of Malachite Green by the Fungus Cunninghamella elegans. Appl. Environ. Microbiol. 67, 4358–4360. doi:10.1128/AEM.67.9.4358-4360.2001

Cheng, Z., Xiang-hua, W., Ping, N., 2013. Continuous Acid Blue 45 decolorization by using a novel open fungal reactor system with ozone as the bactericide. Biochem. Eng. J. 79, 246–252. doi:10.1016/j.bej.2013.08.010

Contreras, S., Rodríguez, M., al Momani, F., Sans, C., Esplugas, S., 2003. Contribution of the ozonation pre-treatment to the biodegradation of aqueous solutions of 2,4-dichlorophenol.

763 Water Res. 37, 3164–71.

764 Cruz-Morató, C., Ferrando-Climent, L., Rodriguez-Mozaz, S., Barceló, D., Marco-Urrea, E.,
765 Vicent, T., Sarrà, M., 2013a. Degradation of pharmaceuticals in non-sterile urban
766 wastewater by Trametes versicolor in a fluidized bed bioreactor. Water Res. 47, 5200–10.
767 doi:10.1016/j.watres.2013.06.007

768 Cruz-Morató, C., Jelić, A., Perez, S., Petrović, M., Barceló, D., Marco-Urrea, E., Sarrà, M.,
769 Vicent, T., 2013b. Continuous treatment of clofibric acid by Trametes versicolor in a
770 fluidized bed bioreactor: Identification of transformation products and toxicity assessment.
771 Biochem. Eng. J. 75, 79–85. doi:10.1016/j.bej.2013.03.020

772 Dai, G., Wang, B., Huang, J., Dong, R., Deng, S., Yu, G., 2015. Occurrence and source
773 apportionment of pharmaceuticals and personal care products in the Beiyun River of
774 Beijing, China. Chemosphere 119, 1033–1039. doi:10.1016/j.chemosphere.2014.08.056

775 Dalgaard, L., Larsen, C., 1999. Metabolism and excretion of citalopram in man: identification of
776 O-acyl- and N-glucuronides. Xenobiotica 29, 1033–1041. doi:10.1080/004982599238092

777 Dashtban, M., Schraft, H., Syed, T.A., Qin, W., 2010. Fungal biodegradation and enzymatic
778 modification of lignin. Int. J. Biochem. Mol. Biol. 1, 36–50.

779 Demoling, F., Figueroa, D., Bååth, E., 2007. Comparison of factors limiting bacterial growth in
780 different soils. Soil Biol. Biochem. 39, 2485–2495. doi:10.1016/j.soilbio.2007.05.002

781 Durairaj, P., Hur, J.-S.S., Yun, H., 2016. Versatile biocatalysis of fungal cytochrome P450
782 monooxygenases. Microb. Cell Fact. 15, 1–16. doi:10.1186/s12934-016-0523-6

783 Ebele, A.J., Abou-Elwafa Abdallah, M., Harrad, S., 2017. Pharmaceuticals and personal care
784 products (PPCPs) in the freshwater aquatic environment. Emerg. Contam. 3, 1–16.
785 doi:10.1016/j.emcon.2016.12.004

786 Eggert, C., Temp, U., Eriksson, K.E., 1996. The ligninolytic system of the white rot fungus
787 Pycnoporus cinnabarinus: purification and characterization of the laccase. Appl. Environ.
788 Microbiol. 62, 1151–8.

789 Ehlers, G.A., Rose, P.D., 2005. Immobilized white-rot fungal biodegradation of phenol and
790 chlorinated phenol in trickling packed-bed reactors by employing sequencing batch
791 operation. Bioresour. Technol. 96, 1264–1275. doi:10.1016/j.biortech.2004.10.015

792 Espinosa-Ortiz, E.J., Rene, E.R., Pakshirajan, K., van Hullebusch, E.D., Lens, P.N.L., 2015.
793 Fungal pelleted reactors in wastewater treatment: applications and perspectives. Chem. Eng.
794 J. 283, 553–571. doi:10.1016/j.cej.2015.07.068

795 Evgenidou, E.N., Konstantinou, I.K., Lambropoulou, D.A., 2015. Occurrence and removal of
796 transformation products of PPCPs and illicit drugs in wastewaters: A review. Sci. Total
797 Environ. 505, 905–926. doi:10.1016/j.scitotenv.2014.10.021

Feng, K.-C., Rou, T.-M., Liu, B.-L., Tzeng, Y.-M., Chang, Y.-N., 2004. Effect of fungal pellet size on the high yield production of destruxin B by Metarhizium anisopliae. Enzyme Microb. Technol. 34, 22–25. doi:10.1016/j.enzmictec.2003.07.006

Fletcher, M., 1994. Bacterial biofilms and biofouling. Curr. Opin. Biotechnol. 5, 302–6.

Frédéric, O., Yves, P., 2014. Pharmaceuticals in hospital wastewater: their ecotoxicity and contribution to the environmental hazard of the effluent. Chemosphere 115, 31–9. doi:10.1016/j.chemosphere.2014.01.016

Fujioka, T., Khan, S.J., McDonald, J.A., Nghiem, L.D., 2014. Ozonation of N-Nitrosamines in the Reverse Osmosis Concentrate from Water Recycling Applications. Ozone Sci. Eng. 36, 174–180. doi:10.1080/01919512.2013.866885

Gao, D., Zeng, Y., Wen, X., Qian, Y., 2008. Competition strategies for the incubation of white rot fungi under non-sterile conditions. Process Biochem. 43, 937–944. doi:10.1016/j.procbio.2008.04.026

Gomes, J., Costa, R., Quinta-Ferreira, R.M., Martins, R.C., 2017. Application of ozonation for pharmaceuticals and personal care products removal from water. Sci. Total Environ. 586, 265–283. doi:10.1016/j.scitotenv.2017.01.216

Grandclément, C., Seyssiecq, I., Piram, A., Wong-Wah-Chung, P., Vanot, G., Tiliacos, N., Roche, N., Doumenq, P., 2017. From the conventional biological wastewater treatment to hybrid processes, the evaluation of organic micropollutant removal: A review. Water Res. 111, 297–317. doi:10.1016/j.watres.2017.01.005

Gros, M., Cruz-Morato, C., Marco-Urrea, E., Longrée, P., Singer, H., Sarrà, M., Hollender, J., Vicent, T., Rodriguez-Mozaz, S., Barceló, D., 2014. Biodegradation of the X-ray contrast agent iopromide and the fluoroquinolone antibiotic ofloxacin by the white rot fungus Trametes versicolor in hospital wastewaters and identification of degradation products. Water Res. 60, 228–241. doi:10.1016/j.watres.2014.04.042

Hai, F.I., Yamamoto, K., Nakajima, F., Fukushi, K., 2009. Factors governing performance of continuous fungal reactor during non-sterile operation - The case of a membrane bioreactor treating textile wastewater. Chemosphere 74, 810–817. doi:10.1016/j.chemosphere.2008.10.025

Hai, F.I., Yamamoto, K., Nakajima, F., Fukushi, K., 2008. Removal of structurally different dyes in submerged membrane fungi reactor - Biosorption/PAC-adsorption, membrane retention and biodegradation. J. Memb. Sci. 325, 395–403. doi:10.1016/j.memsci.2008.08.006

Hai, F.I., Yamamoto, K., Nakajima, F., Fukushi, K., Nghiem, L.D., Price, W.E., Jin, B., 2013. Degradation of azo dye acid orange 7 in a membrane bioreactor by pellets and attached growth of Coriolus versicolour. Bioresour. Technol. 141, 29–34. doi:10.1016/j.biortech.2013.02.020

Harms, H., Schlosser, D., Wick, L.Y., 2011. Untapped potential: exploiting fungi in

bioremediation of hazardous chemicals. Nat. Rev. Microbiol. 9, 177–92. doi:10.1038/nrmicro2519

Harvey, P.J., Floris, R., Lundell, T., Palmer, J.M., Schoemaker, H.E., Wever, R., 1992. Catalytic mechanisms and regulation of lignin peroxidase. Biochem. Soc. Trans. 20, 345–349. doi:10.1042/bst0200345

Hofrichter, M., 2002. Review: Lignin conversion by manganese peroxidase (MnP). Enzyme Microb. Technol. 30, 454–466. doi:10.1016/S0141-0229(01)00528-2

Jelic, A., Cruz-Morató, C., Marco-Urrea, E., Sarrà, M., Perez, S., Vicent, T., Petrović, M., Barcelo, D., 2012. Degradation of carbamazepine by Trametes versicolor in an air pulsed fluidized bed bioreactor and identification of intermediates. Water Res. 46, 1–10. doi:10.1016/j.watres.2011.11.063

Jones, S.M., Solomon, E.I., 2015. Electron transfer and reaction mechanism of laccases. Cell. Mol. Life Sci. 72, 869–883. doi:10.1007/s00018-014-1826-6

Jorgensen, S.E., Halling-Sorensen, B., 2000. Drugs in the environment. Chemosphere 40, 691–699. doi:10.1016/S0045-6535(99)00438-5

Joss, A., Zabczynski, S., Göbel, A., Hoffmann, B., Löffler, D., McArdell, C.S., Ternes, T.A., Thomsen, A., Siegrist, H., 2006. Biological degradation of pharmaceuticals in municipal wastewater treatment: Proposing a classification scheme. Water Res. 40, 1686–1696. doi:10.1016/j.watres.2006.02.014

Kaiser, E., Prasse, C., Wagner, M., Bröder, K., Ternes, T.A., 2014. Transformation of oxcarbazepine and human metabolites of carbamazepine and oxcarbazepine in wastewater treatment and sand filters. Environ. Sci. Technol. 48, 10208–16. doi:10.1021/es5024493

Kamei, I., Kondo, R., 2005. Biotransformation of dichloro-, trichloro-, and tetrachlorodibenzo-p-dioxin by the white-rot fungus Phlebia lindtneri. Appl. Microbiol. Biotechnol. 68, 560–566. doi:10.1007/s00253-005-1947-9

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance. Environ. Sci. Technol. 36, 1202–1211. doi:10.1021/es011055j

Kresinová, Z., Linhartová, L., Filipová, A., Ezechiáš, M., Mašín, P., Cajthaml, T., 2017. Biodegradation of endocrine disruptors in urban wastewater using Pleurotus ostreatus bioreactor. N. Biotechnol. doi:10.1016/j.nbt.2017.05.004

Kusvuran, E., Yildirim, D., 2013. Degradation of bisphenol A by ozonation and determination of degradation intermediates by gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry. Chem. Eng. J. 220, 6–14. doi:10.1016/j.cej.2013.01.064

Leidig, E., Prüsse, U., Vorlop, K.-D., Winter, J., 1999. Biotransformation of Poly R-478 by continuous cultures of PVAL-encapsulated Trametes versicolor under non-sterile conditions. Bioprocess Eng. 21, 5–12. doi:10.1007/PL00009064

Leonowicz, A., Matuszewska, A., Luterek, J., Ziegenhagen, D., Wojtaś-Wasilewska, M., Cho, N.-S., Hofrichter, M., Rogalski, J., 1999. Biodegradation of Lignin by White Rot Fungi. Fungal Genet. Biol. 27, 175–185. doi:10.1006/fgbi.1999.1150

Leštan, D., Lamar, R.T., 1999. Influence of humidity on production of pelleted fungal inoculum. World J. Microbiol. Biotechnol. 15, 349–357. doi:10.1023/A:1008983521015

Li, X., Xu, J., de Toledo, R.A., Shim, H., 2016. Enhanced carbamazepine removal by immobilized Phanerochaete chrysosporium in a novel rotating suspension cartridge reactor under non-sterile condition. Int. Biodeterior. Biodegradation 115, 102–109. doi:10.1016/j.ibiod.2016.08.003

Li, X., Xu, J., de Toledo, R.A., Shim, H., Toledo, R.A. De, Shim, H., 2015. Enhanced removal of naproxen and carbamazepine from wastewater using a novel countercurrent seepage bioreactor immobilized with Phanerochaete chrysosporium under non-sterile conditions. Bioresour. Technol. 197, 465–474. doi:10.1016/j.biortech.2015.08.118

Libra, J.A., Borchert, M., Banit, S., 2003. Competition strategies for the decolorization of a textile-reactive dye with the white-rot fungi Trametes versicolor under non-sterile conditions. Biotechnol. Bioeng. 82, 736–744. doi:10.1002/bit.10623

Liu, X., Li, X.-M., Yang, Q., Yue, X., Shen, T.-T., Zheng, W., Luo, K., Sun, Y.-H., Zeng, G.-M., 2012. Landfill leachate pretreatment by coagulation-flocculation process using iron-based coagulants: Optimization by response surface methodology. Chem. Eng. J. 200–202, 39–51. doi:10.1016/j.cej.2012.06.012

López-Maldonado, E.A., Oropeza-Guzman, M.T., Jurado-Baizaval, J.L., Ochoa-Terán, A., 2014. Coagulation-flocculation mechanisms in wastewater treatment plants through zeta potential measurements. J. Hazard. Mater. 279, 1–10. doi:10.1016/j.jhazmat.2014.06.025

Lu, Y., Yan, L., Wang, Y., Zhou, S., Fu, J., Zhang, J., 2009. Biodegradation of phenolic compounds from coking wastewater by immobilized white rot fungus Phanerochaete chrysosporium. J. Hazard. Mater. 165, 1091–1097. doi:10.1016/j.jhazmat.2008.10.091

Lynn, R.K., Smith, R.G., Thompson, R.M., Deinzer, M.L., Griffin, D., Gerber, N., 1978. Characterization of glucuronide metabolites of carbamazepine in human urine by gas chromatography and mass spectrometry. Drug Metab. Dispos. 6, 494–501.

Marco-Urrea, E., Pérez-Trujillo, M., Blánquez, P., Vicent, T., Caminal, G., 2010a. Biodegradation of the analgesic naproxen by Trametes versicolor and identification of intermediates using HPLC-DAD-MS and NMR. Bioresour. Technol. 101, 2159–2166. doi:10.1016/j.biortech.2009.11.019

Marco-Urrea, E., Pérez-Trujillo, M., Cruz-Morató, C., Caminal, G., Vicent, T., 2010b. White-rot

fungus-mediated degradation of the analgesic ketoprofen and identification of intermediates by HPLC-DAD-MS and NMR. Chemosphere 78, 474–81. doi:10.1016/j.chemosphere.2009.10.009

Marco-Urrea, E., Pérez-Trujillo, M., Cruz-Morató, C., Caminal, G., Vicent, T., 2010c. Degradation of the drug sodium diclofenac by Trametes versicolor pellets and identification of some intermediates by NMR. J. Hazard. Mater. 176, 836–842. doi:10.1016/j.jhazmat.2009.11.112

Marco-Urrea, E., Pérez-Trujillo, M., Vicent, T., Caminal, G., 2009. Ability of white-rot fungi to remove selected pharmaceuticals and identification of degradation products of ibuprofen by Trametes versicolor. Chemosphere 74, 765–772. doi:10.1016/j.chemosphere.2008.10.040

Marinho, G., Barbosa, B.C.A., Rodrigues, K., Aquino, M., Pereira, L., 2017. Potential of the filamentous fungus Aspergillus niger AN 400 to degrade Atrazine in wastewaters. Biocatal. Agric. Biotechnol. 9, 162–167. doi:10.1016/j.bcab.2016.12.013

Mir-Tutusaus, J.A., Caminal, G., Sarrà, M., in press. Influence of process variables in a continuous treatment of non-sterile hospital wastewater by Trametes versicolor and novel method for inoculum production. J. Environ. Manage. doi:10.1016/j.jenvman.2018.02.018

Mir-Tutusaus, J.A., Massís-Mora, M., Corcellas, C., Eljarrat, E., Barceló, D., Sarrà, M., Caminal, G., Vicent, T., Rodríguez-Rodríguez, C.E., 2014. Degradation of selected agrochemicals by the white rot fungus Trametes versicolor. Sci. Total Environ. 500–501, 235–242. doi:10.1016/j.scitotenv.2014.08.116

Mir-Tutusaus, J.A., Parladé, E., Llorca, M., Villagrasa, M., Barceló, D., Rodriguez-Mozaz, S., Martinez-Alonso, M., Gaju, N., Caminal, G., Sarrà, M., 2017. Pharmaceuticals removal and microbial community assessment in a continuous fungal treatment of non-sterile real hospital wastewater after a coagulation-flocculation pretreatment. Water Res. 116, 65–75. doi:10.1016/j.watres.2017.03.005

Mir-Tutusaus, J.A., Sarrà, M., Caminal, G., 2016. Continuous treatment of non-sterile hospital wastewater by Trametes versicolor? How to increase fungal viability by means of operational strategies and pretreatments. J. Hazard. Mater. 318, 561–570. doi:10.1016/j.jhazmat.2016.07.036

Morozova, O. V., Shumakovich, G.P., Shleev, S. V., Yaropolov, Y.I., 2007. Laccase-mediator systems and their applications: A review. Appl. Biochem. Microbiol. 43, 523–535. doi:10.1134/S0003683807050055

Naylor, C.G., 1995. Environmental fate and safety of nonylphenol ethoxylates. Surfactants and Wastewater 27, 29–33.

Nerud, F., Misurcova, Z., 1996. Distribution of Ligninolytic Enzymes in Selected White-Rot Fungi. Folia Microbiol. 41, 1988–1990. doi:10.1007/BF02814628

Nguyen, L.N., Hai, F.I., Yang, S., Kang, J., Leusch, F.D.L., Roddick, F., Price, W.E., Nghiem,

L.D., 2013. Removal of trace organic contaminants by an MBR comprising a mixed culture of bacteria and white-rot fungi. Bioresour. Technol. 148, 234–241. doi:10.1016/j.biortech.2013.08.142

Nilsson, I., Möller, A., Mattiasson, B., Rubindamayugi, M.S.T., Welander, U., 2006. Decolorization of synthetic and real textile wastewater by the use of white-rot fungi. Enzyme Microb. Technol. 38, 94–100. doi:10.1016/j.enzmictec.2005.04.020

Nogueira, V., Lopes, I., Freitas, A.C., Rocha-Santos, T.A.P., Gonçalves, F., Duarte, A.C., Pereira, R., 2015. Biological treatment with fungi of olive mill wastewater pre-treated by photocatalytic oxidation with nanomaterials. Ecotoxicol. Environ. Saf. 115, 234–242. doi:10.1016/j.ecoenv.2015.02.028

Ntougias, S., Baldrian, P., Ehaliotis, C., Nerud, F., Merhautová, V., Zervakis, G.I., 2015. Olive mill wastewater biodegradation potential of white-rot fungi - Mode of action of fungal culture extracts and effects of ligninolytic enzymes. Bioresour. Technol. 189, 121–130. doi:10.1016/j.biortech.2015.03.149

Palli, L., Castellet-Rovira, F., Pérez-Trujillo, M., Caniani, D., Sarrà-Adroguer, M., Gori, R., 2017. Preliminary evaluation of Pleurotus ostreatus for the removal of selected pharmaceuticals from hospital wastewater. Biotechnol. Prog. doi:10.1002/btpr.2520

Palli, L., Gullotto, A., Tilli, S., Caniani, D., Gori, R., Scozzafava, A., 2016. Biodegradation of 2-naphthalensulfonic acid polymers by white-rot fungi: Scale-up into non-sterile packed bed bioreactors. Chemosphere 164, 120–127. doi:10.1016/j.chemosphere.2016.08.071

Pazarlioglu, N.K., Sariisik, M., Telefoncu, A., 2005. Laccase: Production by Trametes versicolor and application to denim washing. Process Biochem. 40, 1673–1678. doi:10.1016/j.procbio.2004.06.052

Pedroza-Rodríguez, A.M., Rodríguez-Vázquez, R., 2013. Optimization of C/N Ratio and Inducers for Wastewater Paper Industry Treatment Using Trametes versicolor Immobilized in Bubble Column Reactor. J. Mycol. 2013, 1–11. doi:10.1155/2013/536721

Pointing, S.B., 2001. Feasibility of bioremediation by white-rot fungi. Appl. Microbiol. Biotechnol. 57, 20–33. doi:10.1007/s002530100745

Quintero, J.C., Lú-Chau, T.A., Moreira, M.T., Feijoo, G., Lema, J.M., 2007. Bioremediation of HCH present in soil by the white-rot fungus Bjerkandera adusta in a slurry batch bioreactor. Int. Biodeterior. Biodegrad. 60, 319–326. doi:10.1016/j.ibiod.2007.05.005

Ramos, V., Bocalandro, C., Riquelme, S., Sanhueza, V., Aspé, E., Roeckel, M., Fernández, K., 2013. Effect of the bench scale extraction conditions on Pinus radiata bark extract yield, antioxidant properties and composition. Maderas. Cienc. y Tecnol. 15, 0–0. doi:10.4067/S0718-221X2013005000003

Reddy, C.A., 1995. The potential for white-rot fungi in the treatment of pollutants. Curr. Opin. Biotechnol. 6, 320–328. doi:10.1016/0958-1669(95)80054-9

Rodarte-Morales, A.I., Feijoo, G., Moreira, M.T., Lema, J.M., 2011. Degradation of selected pharmaceutical and personal care products (PPCPs) by white-rot fungi. World J. Microbiol. Biotechnol. 27, 1839–1846. doi:10.1007/s11274-010-0642-x

Rodarte-morales, A.I., Feijoo, G., Moreira, M.T., Lema, J.M., Lema, M., 2012. Evaluation of Two Operational Regimes: Fed-Batch and Continuous for the Removal of Pharmaceuticals in a Fungal Stirred Tank Reactor. Chem. Eng. Trans. 27, 151–156. doi:10.3303/CET1227026

Rodarte-Morales, A.I.I., Feijoo, G., Moreira, M.T.T., Lema, J.M.M., 2012. Operation of stirred tank reactors (STRs) and fixed-bed reactors (FBRs) with free and immobilized Phanerochaete chrysosporium for the continuous removal of pharmaceutical compounds. Biochem. Eng. J. 66, 38–45. doi:10.1016/j.bej.2012.04.011

Rodríguez-Rodríguez, C.E., Jesús García-Galán, M., Blánquez, P., Díaz-Cruz, M.S., Barceló, D., Caminal, G., Vicent, T., 2012. Continuous degradation of a mixture of sulfonamides by Trametes versicolor and identification of metabolites from sulfapyridine and sulfathiazole. J. Hazard. Mater. 213–214, 347–354. doi:10.1016/j.jhazmat.2012.02.008

Rodríguez Couto, S., Rodríguez, A., Paterson, R.R.M., Lima, N., Teixeira, J.A., 2006. Laccase activity from the fungus Trametes hirsuta using an air-lift bioreactor. Lett. Appl. Microbiol. 42, 612–616. doi:10.1111/j.1472-765X.2006.01879.x

Rousk, J., Bååth, E., 2007. Fungal and bacterial growth in soil with plant materials of different C/N ratios. FEMS Microbiol. Ecol. 62, 258–267. doi:10.1111/j.1574-6941.2007.00398.x

Rubirola, A., Llorca, M., Rodriguez-Mozaz, S., Casas, N., Rodriguez-Roda, I., Barceló, D., Buttiglieri, G., 2014. Characterization of metoprolol biodegradation and its transformation products generated in activated sludge batch experiments and in full scale WWTPs. Water Res. 63, 21–32. doi:10.1016/j.watres.2014.05.031

Ruiz-Duenas, F.J., Martinez, M.J., Martinez, A.T., 1999. Molecular characterization of a novel peroxidase isolated from the ligninolytic fungus Pleurotus eryngii. Mol. Microbiol. 31, 223–235. doi:10.1046/j.1365-2958.1999.01164.x

Sankaran, S., Khanal, S.K., Pometto, A.L., van Leeuwen, J. (Hans), 2008. Ozone as a selective disinfectant for nonaseptic fungal cultivation on corn-processing wastewater. Bioresour. Technol. 99, 8265–8272. doi:10.1016/j.biortech.2008.03.055

Sauvé, S., Desrosiers, M., 2014. A review of what is an emerging contaminant. Chem. Cent. J. 8, 15. doi:10.1186/1752-153X-8-15

Sharma, A., Padwal-Desai, S.R., 1985. On the relationship between pellet size and aflatoxin yield in Aspergillus parasiticus. Biotechnol. Bioeng. 27, 1577–1580. doi:10.1002/bit.260271109

Shreve, M.J., Brockman, A., Hartleb, M., Prebihalo, S., Dorman, F.L., Brennan, R.A., 2016. The white-rot fungus Trametes versicolor reduces the estrogenic activity of a mixture of

emerging contaminants in wastewater treatment plant effluent. Int. Biodeterior. Biodegrad. 109, 132–140. doi:10.1016/j.ibiod.2016.01.018

Sirtori, C., Zapata, A., Oller, I., Gernjak, W., Agüera, A., Malato, S., Agu, A., 2009. Decontamination industrial pharmaceutical wastewater by combining solar photo-Fenton and biological treatment. Water Res. 43, 661–668. doi:10.1016/j.watres.2008.11.013

Sitanggang, A.B., Wu, H.-S., Wang, S.S., Ho, Y.-C., 2010. Effect of pellet size and stimulating factor on the glucosamine production using Aspergillus sp. BCRC 31742. Bioresour. Technol. 101, 3595–3601. doi:10.1016/j.biortech.2009.12.084

Spiker, J.K., Crawford, D.L., Crawford, R.L., 1992. Influence of 2,4,6-trinitrotoluene (TNT) concentration on the degradation of TNT in explosive-contaminated soils by the white rot fungus Phanerochaete chrysosporium. Appl. Environ. Microbiol.

Stojan, J., Komel, R., Jawallapersand, P., Mashele, S.S., Kovac, L., Syed, K., Pakala, S.B., Kras, N., Kovačič, L., Stojan, J., Komel, R., Pakala, S.B., Kraševec, N., Syed, K., Jawallapersand, P., Mashele, S.S., Kovac, L., Syed, K., Pakala, S.B., Kras, N., 2014. Cytochrome P450 monooxygenase CYP53 family in fungi: Comparative structural and evolutionary analysis and its role as a common alternative anti-fungal drug target. PLoS One 9, e107209. doi:10.1371/journal.pone.0107209

Tang, W., Jia, R., Zhang, D., 2011. Decolorization and degradation of synthetic dyes by Schizophyllum sp. F17 in a novel system. Desalination 265, 22–27. doi:10.1016/j.desal.2010.07.024

Ternes, T.A., Stüber, J., Herrmann, N., McDowell, D., Ried, A., Kampmann, M., Teiser, B., 2003. Ozonation: a tool for removal of pharmaceuticals, contrast media and musk fragrances from wastewater? Water Res. 37, 1976–1982. doi:10.1016/S0043-1354(02)00570-5

Torán, J., Blánquez, P., Caminal, G., 2017. Comparison between several reactors with Trametes versicolor immobilized on lignocellulosic support for the continuous treatments of hospital wastewater. Bioresour. Technol. 243, 966–974. doi:10.1016/j.biortech.2017.07.055

Trueba-Santiso, A., Parladé, E., Rosell, M., Lliros, M., Mortan, S.H., Martínez-Alonso, M., Gaju, N., Martín-González, L., Vicent, T., Marco-Urrea, E., 2017. Molecular and carbon isotopic characterization of an anaerobic stable enrichment culture containing Dehalobacterium sp. during dichloromethane fermentation. Sci. Total Environ. 581–582, 640–648. doi:10.1016/J.SCITOTENV.2016.12.174

Valentín, L., Lu-Chau, T.A., López, C., Feijoo, G., Moreira, M.T., Lema, J.M., 2007. Biodegradation of dibenzothiophene, fluoranthene, pyrene and chrysene in a soil slurry reactor by the white-rot fungus Bjerkandera sp. BOS55. Process Biochem. 42, 641–648. doi:10.1016/j.procbio.2006.11.011

Van Driessel, B., Christov, L., 2001. Decolorization of bleach plant effluent by mucoralean and

white-rot fungi in a rotating biological contactor reactor. J. Biosci. Bioeng. 92, 271–276. doi:10.1016/S1389-1723(01)80261-9

van Leeuwen, J.H., Hu, Z., Yi, T., Pometto, A.L.I., Jin, B., 2003. Kinetic Model for Selective Cultivation of Microfungi in a Microscreen Process for Food Processing Wastewater Treatment and Biomass Production. Acta Biotechnol. 23, 289–300. doi:10.1002/abio.200390036

Verlicchi, P., Al Aukidy, M., Zambello, E., 2015. What have we learned from worldwide experiences on the management and treatment of hospital effluent? - An overview and a discussion on perspectives. Sci. Total Environ. 514, 467–491. doi:10.1016/j.scitotenv.2015.02.020

Verlicchi, P., Al Aukidy, M., Zambello, E., 2012. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment - A review. Sci. Total Environ. 429, 123–155. doi:10.1016/j.scitotenv.2012.04.028

Verlicchi, P., Galletti, A., Petrovic, M., Barceló, D., 2010. Hospital effluents as a source of emerging pollutants: An overview of micropollutants and sustainable treatment options. J. Hydrol. 389, 416–428. doi:10.1016/j.jhydrol.2010.06.005

Viswanath, B., Rajesh, B., Janardhan, A., Kumar, A.P., Narasimha, G., 2014. Fungal laccases and their applications in bioremediation. Enzyme Res. 2014, 1–21. doi:10.1155/2014/163242

Wang, D., Sui, Q., Lu, S.G., Zhao, W.T., Qiu, Z.F., Miao, Z.W., Yu, G., 2014. Occurrence and removal of six pharmaceuticals and personal care products in a wastewater treatment plant employing anaerobic/anoxic/aerobic and UV processes in Shanghai, China. Environ. Sci. Pollut. Res. 21, 4276–4285. doi:10.1007/s11356-013-2363-9

Wen, J., Gao, D., Zhang, B., Liang, H., 2011. Co-metabolic degradation of pyrene by indigenous white-rot fungus Pseudotrametes gibbosa from the northeast China. Int. Biodeterior. Biodegradation 65, 600–604. doi:10.1016/j.ibiod.2011.03.003

Wittier, R., Baumgartl, H., Lübbers, D.W., Schügerl, K., 1986. Investigations of oxygen transfer intoPenicillium chrysogenum pellets by microprobe measurements. Biotechnol. Bioeng. 28, 1024–1036. doi:10.1002/bit.260280713

World Health Organization, 2016. WHO | Antibiotic resistance. WHO 1–11.

Xiao, P., Mori, T., Kamei, I., Kondo, R., 2011. A novel metabolic pathway for biodegradation of DDT by the white rot fungi, Phlebia lindtneri and Phlebia brevispora. Biodegradation 22, 859–867. doi:10.1007/s10532-010-9443-z

Yadav, J.S., Reddy, C.A., 1993. Degradation of benzene, toluene, ethylbenzene, and xylenes (BTEX) by the lignin-degrading basidiomycete Phanerochaete chrysosporium. Appl. Environ. Microbiol. 59, 756–762.

Yadav, M., Srivastva, N., Shukla, A.K., Singh, R.S., Upadhyay, S.N., Dubey, S.K., 2015. Efficacy of Aspergillus sp. for Degradation of Chlorpyrifos in Batch and Continuous Aerated Packed Bed Bioreactors. Appl. Biochem. Biotechnol. 175, 16–24. doi:10.1007/s12010-014-1244-0

Yang, J., Li, J., Dong, W., Ma, J., Cao, J., Li, T., Li, J., Gu, J., Liu, P., 2016. Study on enhanced degradation of atrazine by ozonation in the presence of hydroxylamine. J. Hazard. Mater. 316, 110–121. doi:10.1016/j.jhazmat.2016.04.078

Yang, S., Hai, F.I., Nghiem, L.D., Nguyen, L.N., Roddick, F., Price, W.E., 2013. Removal of bisphenol A and diclofenac by a novel fungal membrane bioreactor operated under non-sterile conditions. Int. Biodeterior. Biodegrad. 85, 483–490. doi:10.1016/j.ibiod.2013.03.012

Yang, Y., Ok, Y.S., Kim, K., Kwon, E.E., Tsang, Y.F., 2017. Occurrences and removal of pharmaceuticals and personal care products (PPCPs) in drinking water and water/sewage treatment plants: A review. Sci. Total Environ. 596–597, 303–320. doi:10.1016/j.scitotenv.2017.04.102

Zhang, Y., Geißen, S.U., 2012. Elimination of carbamazepine in a non-sterile fungal bioreactor. Bioresour. Technol. 112, 221–227. doi:10.1016/j.biortech.2012.02.073

Zhuo, R., Ma, L., Fan, F., Gong, Y., Wan, X., Jiang, M., Zhang, X., Yang, Y., 2011. Decolorization of different dyes by a newly isolated white-rot fungi strain Ganoderma sp.En3 and cloning and functional analysis of its laccase gene. J. Hazard. Mater. 192, 855–873. doi:10.1016/j.jhazmat.2011.05.106

Table 1. Removal efficiencies of fungal systems for pharmaceutically active compounds.

Compound	Fungus	Duration of the treatment	Reactor	HRT	Matrix	pH	Sterility	Initial concentration		Spiked matrix	Removal (%)	Source
Analgesics and anti-inflammatories												
Acetaminophen	*T. versicolor*	56 d	FBR	3 d	Flocculated HWW	4.5	No	> 20000	ng·L-1	No	> 99.3	Mir-Tutusaus et al. 2017
Diclofenac	*P. chrysosporium*	30 d	stirred tank	24 h	Kirk medium	4.5	Yes	1	mg·L-1	Yes	100	Rodarte-Morales et al. 2011
	P. chrysosporium	50 d	stirred tank	24 h	Kirk medium	4.5	Yes	1	mg·L-1	Yes	>93	Rodarte-Morales et al. 2012
	P. chrysosporium	100 d	fixed bed	24 h	Kirk medium	4.5	Yes	1	mg·L-1	Yes	100	Rodarte-Morales et al. 2012
	P. chrysosporium	70 d	stirred tank	-	-	3.7-5.3	Yes	0.9-1.7	mg·L-1	Yes	34-90	Rodarte-Morales et al. 2012b
	T. versicolor	90 d	MBR	48 h	Malt extract-based	5,4	No	300-1500	µg·L-1	Yes	0-60	Yang et al. 2013
	T. versicolor	110 d	MBR	2 d	Malt extract-based	4.5	No	5	µg·L-1	Yes	50	Nguyen et al. 2013
	T. versicolor	26 d	FBR	3.3 d	Veterinary HWW	4.5	No	123	ng·L-1	No	-177	Badia-Fabregat et al. 2015b
	T. versicolor	56 d	FBR	3 d	Flocculated HWW	4.5	No	951	ng·L-1	No	99,8	Mir-Tutusaus et al. 2017
Ibuprofen	*P. chrysosporium*	30 d	stirred tank	24 h	Kirk medium	4.5	Yes	1	mg·L-1	Yes	100	Rodarte-Morales et al. 2011
	P. chrysosporium	50 d	stirred tank	24 h	Kirk medium	4.5	Yes	1	mg·L-1	Yes	>93	Rodarte-Morales et al. 2012
	P. chrysosporium	100 d	fixed bed	24 h	Kirk medium	4.5	Yes	1	mg·L-1	Yes	100	Rodarte-Morales et al. 2012
	P. chrysosporium	70 d	stirred tank	-	-	3.7-5.3	Yes	0.8-1.2	mg·L-1	Yes	65-96	Rodarte-Morales et al. 2012b
	T. versicolor	110 d	MBR	2 d	Malt extract-based	4.5	No	5	µg·L-1	Yes	>95	Nguyen et al. 2013
	T. versicolor	26 d	FBR	3.3 d	Veterinary HWW	4.5	No	212	ng·L-1	No	30	Badia-Fabregat et al. 2015b
	T. versicolor	28 d	FBR	3 d	Flocculated HWW	4.5	No	20	mg·L-1	Yes	100	Mir-Tutusaus et al. 2016
	T. versicolor	56 d	FBR	3 d	Flocculated HWW	4.5	No	>20000	ng·L-1	No	> 85.5	Mir-Tutusaus et al. 2017
Indomethacine	*T. versicolor*	26 d	FBR	3.3 d	Veterinary HWW	4.5	No	34	ng·L-1	No	-79	Badia-Fabregat et al. 2015b
Ketoprofen	*T. versicolor*	110 d	MBR	2 d	Malt extract-based	4.5	No	5	µg·L-1	Yes	94	Nguyen et al. 2013
	T. versicolor	26 d	FBR	3.3 d	Veterinary HWW	4.5	No	320	ng·L-1	No	-57	Badia-Fabregat et al. 2015b
	T. versicolor	28 d	FBR	3 d	Flocculated HWW	4.5	No	20	mg·L-1	Yes	80	Mir-Tutusaus et al. 2016
	T. versicolor	56 d	FBR	3 d	Flocculated HWW	4.5	No	5109	ng·L-1	No	-3,6	Mir-Tutusaus et al. 2017
Naproxen	*P. chrysosporium*	30 d	stirred tank	24 h	Kirk medium	4.5	Yes	1	mg·L-1	Yes	83	Rodarte-Morales et al. 2011
	P. chrysosporium	50 d	stirred tank	24 h	Kirk medium	4.5	Yes	1	mg·L-1	Yes	0-92	Rodarte-Morales et al. 2012
	P. chrysosporium	100 d	fixed bed	24 h	Kirk medium	4.5	Yes	1	mg·L-1	Yes	90	Rodarte-Morales et al. 2012
	P. chrysosporium	70 d	stirred tank	-	-	3.7-5.3	Yes	0.9-1.3	mg·L-1	Yes	0-94	Rodarte-Morales et al. 2012b
	T. versicolor	110 d	MBR	2 d	Malt extract-based	4.5	No	5	µg·L-1	Yes	>99	Nguyen et al. 2013
	T. versicolor	26 d	FBR	3.3 d	Veterinary HWW	4.5	No	85	ng·L-1	No	71	Badia-Fabregat et al. 2015b
	P. chrysosporium	165 d	seepage reactor	2 d	Kirk medium	4.5	No	1	mg·L-1	Yes	100	Li et al. 2015
Piroxicam	*T. versicolor*	26 d	FBR	3.3 d	Veterinary HWW	4.5	No	136	ng·L-1	No	-59	Badia-Fabregat et al. 2015b
Salicyclic acid	*T. versicolor*	110 d	MBR	2 d	Malt extract-based	4.5	No	5	µg·L-1	Yes	90	Nguyen et al. 2013
	T. versicolor	26 d	FBR	3.3 d	Veterinary HWW	4.5	No	3730	ng·L-1	No	81	Badia-Fabregat et al. 2015b
Anthelmintics												
Albendazole	*T. versicolor*	26 d	FBR	3.3 d	Veterinary HWW	4.5	No	8	ng·L-1	No	45	Badia-Fabregat et al. 2015b
Thiabendazole	*T. versicolor*	56 d	FBR	3 d	Flocculated HWW	4.5	No	blq	ng·L-1	No	70,0	Mir-Tutusaus et al. 2017
Antibiotics												
Ciprofloxacin	*T. versicolor*	56 d	FBR	3 d	Flocculated HWW	4.5	No	366	ng·L-1	No	47,1	Mir-Tutusaus et al. 2017
	T. versicolor	26 d	FBR	3.3 d	Veterinary HWW	4.5	No	42	ng·L-1	No	100	Badia-Fabregat et al. 2015b
Metronidazole	*T. versicolor*	110 d	MBR	2 d	Malt extract-based	4.5	No	5	µg·L-1	Yes	38	Nguyen et al. 2013
	T. versicolor	26 d	FBR	3.3 d	Veterinary HWW	4.5	No	1736	ng·L-1	No	40	Badia-Fabregat et al. 2015b
Ofloxacin	*T. versicolor*	8 d	FBR	batch	HWW	4.5	No	202	µg·L-1	No	99	Gros et al. 2014
	T. versicolor	8 d	FBR	batch	HWW	4.5	Yes	32	µg·L-1	No	98,5	Gros et al. 2014
	T. versicolor	56 d	FBR	3 d	Flocculated HWW	4.5	No	2537	ng·L-1	No	71,1	Mir-Tutusaus et al. 2017
Ronidazole	*T. versicolor*	56 d	FBR	3 d	Flocculated HWW	4.5	No	bld	ng·L-1	No	-7745,2	Mir-Tutusaus et al. 2017
Sulfamethazine	*T. versicolor*	26 d	FBR	3 d	Defined medium	4.5	Yes	5	mg·L-1	Yes	>94	Rodriguez-Rodriguez et al. et al. 20
Sulfamethoxazole	*T. versicolor*	56 d	FBR	3 d	Flocculated HWW	4.5	No	1130	ng·L-1	No	78,2	Mir-Tutusaus et al. 2017
Sulfapyridine	*T. versicolor*	26 d	FBR	3 d	Defined medium	4.5	Yes	5	mg·L-1	Yes	>99	Rodriguez-Rodriguez et al. 2012
Sulfathiazole	*T. versicolor*	26 d	FBR	3 d	Defined medium	4.5	Yes	5	mg·L-1	Yes	>95	Rodriguez-Rodriguez et al. 2012
Trimethoprim	*T. versicolor*	56 d	FBR	3 d	Flocculated HWW	4.5	No	748	ng·L-1	No	52,3	Mir-Tutusaus et al. 2017
Anticoagulants												
Warfarin	*T. versicolor*	56 d	FBR	3 d	Flocculated HWW	4.5	No	10	ng·L-1	No	94,8	Mir-Tutusaus et al. 2017
Antihypertensives												
Valsartan	*T. versicolor*	56 d	FBR	3 d	Flocculated HWW	4.5	No	113	ng·L-1	No	34,2	Mir-Tutusaus et al. 2017

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1121Table 2. Removal efficiencies of fungal systems for endocrine disruptors.

Table 2. Removal efficiencies of fungal systems for pesticides and industrial chemicals.

Compound	Fungus	Duration of the treatment	Duration of the treatment	Reactor	HRT	HRT	Matrix	pH	Sterility	pH	Sterility	Initial concentration	Initial concentration	Spiked matrix	Removal (%)	Source	Removal (%)	Source
Endocrine disruptors																		
17α-ethinylestradiol (EE2)	*T. versicolor*	10 d	MBR	10 d	MBR	120 h	Defined medium	4.5	No	7.3		ng L-1	Yes	pg L-1	Yes	97	Blánquez et al. 2004	Blánquez et al. 2013
Atrazine	*T. versicolor*	10 d	MBR	10 d	MBR	2 d	Malt extract-based	4.5	No	5		µg L-1	Yes	µg L-1	Yes	90	Mgayen et al. 2013	Mgayen et al. 2013
	T. versicolor	12 h	bottle reactor	12 h	bottle reactor	batch	WWTP effluent	4.5	Yes	350		µg L-1	Yes	µg L-1	Yes	71.3	Shreve et al. 2016	Shreve et al. 2016
	P. ostreatus	28 d	trickle bed	28 d	trickle bed	48 h	WWTP effluent	3-5	Yes	10		mg L-1	No	mg L-1	Yes	50	Fernández-Fontaina et al. 2017	Fernández-Fontaina et al. 2017
17β-estradiol (E2)	*T. versicolor*	*Aspergillus* sp.	26 d	FBR	45 d	packed bed	120 h	Defined medium	4.5	Yes	7	Yes	3-18.8	ng L-1	Yes	99	Blánquez et al. 2004	Blánquez et al. 2014
Clofibric acid	*T. versicolor*	10 d	MBR	10 d	MBR	2 d	Malt extract-based	4.5	No	5		µg L-1	Yes	µg L-1	Yes	99	Mgayen et al. 2013	Mgayen et al. 2013
	T. versicolor	12 h	bottle reactor	12 h	bottle reactor	batch	WWTP effluent	4.5	Yes	350		µg L-1	Yes	µg L-1	Yes	99	Shreve et al. 2016	Rodríguez-Narvaez et al. 2013
17β-estradiol 3-acetate	*T. versicolor*	10 d	MBR	10 d	MBR	2 d	Malt extract-based	4.5	No	5		µg L-1	Yes	µg L-1	Yes	95	Mgayen et al. 2013	Mgayen et al. 2013
4-n-nonylphenol (NP)	*P. ostreatus*	*T. versicolor*	28 d	trickle bed	28 d	trickle bed	48 h	WWTP effluent	7.2-8	Yes	10		ng L-1	No	µg L-1	Yes	50	Fernández-Fontaina et al. 2016	Fernández-Fontaina et al. 2016
Bisphenol A (BPA)	*T. versicolor*	10 d	MBR	10 d	MBR	48 h	Malt extract-based	4.5	No	300-1500		µg L-1	Yes	µg L-1	Yes	90	Yang et al. 2012	Mgayen et al. 2013
Propamocarb	*T. versicolor*	10 d	MBR	10 d	MBR	2 d	Malt extract-based	4.5	No	5		µg L-1	Yes	µg L-1	Yes	75	Mgayen et al. 2013	Mgayen et al. 2013
α-Hexachlorocyclohexane (α-HCH)	*T. versicolor*	*Bjerkandera adusta*	12 h	bottle reactor	12 h	bottle reactor	batch	WWTP effluent	4.5	Yes	350		µg L-1	Yes	ng kg-1	Yes	61.9	Shreve et al. 2016	Marco-Urrea et al. 2007
β-Hexachlorocyclohexane (β-HCH)	*P. ostreatus*	*Bjerkandera adusta*	28 d	trickle bed	30 d	slurry reactor	48 h	WWTP effluent	7.2-8	Yes	20		ng L-1	No	mg kg-1	Yes	80	Fernández-Fontaina et al. 2016	Marco-Urrea et al. 2007
Estriol (E3)	*T. versicolor*	*Bjerkandera adusta*	10 d	MBR	30 d	slurry reactor	2 d	Malt extract-based	4.5	Yes	5		µg L-1	Yes	ng kg-1	Yes	95	Mgayen et al. 2013	Marco-Urrea et al. 2007
Estrone (E1)	*T. versicolor*	*Bjerkandera adusta*	10 d	MBR	30 d	slurry reactor	2 d	Malt extract-based	4.5	Yes	5		µg L-1	Yes	ng kg-1	Yes	94	Mgayen et al. 2013	Marco-Urrea et al. 2007
Carbamazepine	*T. versicolor*		12 h	bottle reactor		batch	WWTP effluent	4.5	Yes	350		µg L-1	Yes			45.6	Shreve et al. 2016	
Diclofenac	*T. versicolor*	*Trametes hirsuta*	12 h	bottle reactor	12 h	bottle reactor	batch	WWTP effluent	4.5	Yes	350		µg L-1	Yes			0	Shreve et al. 2016	Marco-Urrea et al. 2010
Triclosan	*T. versicolor*	*Trametes pubescens*	10 d	MBR	90 d	packed bed	2 d	Malt extract-based	4.5	No	6	No	350	µg L-1	Yes	µg L-1	No	95	Mgayen et al. 2013	Mgayen et al. 2013
4-tert-Butylphenol	*T. versicolor*		12 h	bottle reactor	12 h	bottle reactor	batch	WWTP effluent	4.5	Yes	350		µg L-1	Yes	µg L-1	Yes	0	Shreve et al. 2016	Mgayen et al. 2013
	P. ostreatus	*T. versicolor*	28 d	trickle bed	28 d	trickle bed	48 h	WWTP effluent	7.2-8	Yes	25		ng L-1	No	µg L-1	Yes	34	Fernández-Fontaina et al. 2016	Fernández-Fontaina et al. 2013
UV filters																			
Benzophenone	*T. versicolor*	10 d	MBR	10 d	MBR	2 d	Malt extract-based	4.5	No	5		µg L-1	Yes			85	Mgayen et al. 2013	
Octocrylene	*T. versicolor*	10 d	MBR	10 d	MBR	2 d	Malt extract-based	4.5	No	5		µg L-1	Yes			90	Mgayen et al. 2013	
Oxybenzone	*T. versicolor*	10 d	MBR	10 d	MBR	2 d	Malt extract-based	4.5	No	5		µg L-1	Yes			96	Mgayen et al. 2013	
	T. versicolor	12 h	bottle reactor	12 h	bottle reactor	batch	WWTP effluent	4.5	Yes	350		µg L-1	Yes			0	Shreve et al. 2016	