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Removal of pharmaceuticals, steroid hormones, phytoestrogens, UV-filters, industrial chemicals and pesticides by *Trametes versicolor*: role of biosorption and biodegradation

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Removal of pharmaceuticals, steroid hormones, phytoestrogens, UV-filters, industrial chemicals and pesticides by *Trametes versicolor*: role of biosorption and biodegradation

Abstract

This study reports the removal of 30 diverse trace organic contaminants (TrOC) by live (biosorption and biodegradation), intracellular enzyme-inhibited and chemically inactivated (biosorption only) whole-cell preparations and the fungal extracellular enzyme extract (predominantly laccase) from *Trametes versicolor* (ATCC 7731). Because phenolic substrates are amenable to degradation by laccase, all 14 phenolic TrOC were readily biodegraded. On the other hand, only eight of the 16 non-phenolic TrOC were readily biodegraded while the removal of hydrophilic TrOC ($\log D < 3$) was negligible. With the exception of diclofenac, no non-phenolic TrOC were degraded by the extracellular enzyme extract. The whole-cell culture showed considerably higher degradation of at least seven compounds, indicating the importance of biosorption and subsequent degradation by intracellular and/or mycelium associated enzymes. Improvement (20-90%) of enzymatic degradation of four phenolic and seven non-phenolic TrOC was achieved in the presence of a redox mediator. Compared with the whole-cell culture, mediator-amended extracellular extract achieved better removal of six TrOC, but lower removal of six others. A particular concern was the increased toxicity of the treated media when the redox-mediator was used. In addition to reporting the white-rot fungal removal of two UV filters, three phytoestrogens and a few other pharmaceutically active TrOC for the first time, this study provides unique insights into their removal mechanisms.

Keywords

filters, industrial, chemicals, pesticides, trametes, versicolor, role, biosorption, biodegradation, pharmaceuticals, removal, uv, phytoestrogens, hormones, steroid

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**Removal of pharmaceuticals, steroid hormones, phytoestrogens,
UV-filters, industrial chemicals and pesticides by *Trametes
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Highlights

- Of the 30 TrOC, all 14 phenolic and 8 non-phenolic TrOC were effectively biodegraded
- Most hydrophilic ($\log D < 3$) TrOC were poorly biodegraded
- Intracellular/mycelium-associated enzyme appeared particularly important for 5 TrOC
- Whole-cell performed better than 'enzyme-mediator' system for some but not all TrOC
- UV filters and phytoestrogens were well degraded only when they had phenolic moiety

Abstract

This study reports the removal of 30 diverse trace organic contaminants (TrOC) by live (biosorption + biodegradation), intracellular enzyme-inhibited and chemically inactivated (biosorption only) whole-cell preparations and the fungal extracellular enzyme extract (predominantly laccase) from *Trametes versicolor* (ATCC 7731). Because phenolic substrates are amenable to degradation by laccase, all 14 phenolic TrOC were readily biodegraded. On the other hand, only eight of the 16 non-phenolic TrOC were readily biodegraded while the removal of hydrophilic TrOC ($\log D < 3$) was negligible. With the exception of diclofenac, no non-phenolic TrOC were degraded by the extracellular enzyme extract. The whole-cell culture showed considerably higher degradation of at least seven compounds, indicating the importance of biosorption and subsequent degradation by intracellular and/or mycelium associated enzymes. Improvement (20-90%) of enzymatic degradation of four phenolic and seven non-phenolic TrOC was achieved in the presence of a redox mediator. Compared with the whole-cell culture, mediator-amended extracellular extract achieved better removal of six TrOC, but lower removal of six others. A particular concern was the increased toxicity of the treated media when the redox-mediator was used. In addition to reporting the white-rot fungal removal of two UV filters, three phytoestrogens and a few other pharmaceutically active TrOC for the first time, this study provides unique insights into their removal mechanisms.

Keywords: white-rot fungus; trace organic contaminants (TrOC); crude enzyme extract; extracellular enzyme independent degradation; redox mediator

1. Introduction

Trace organic contaminants (TrOC) consist of both naturally occurring and anthropogenic compounds and are frequently detected in sewage-impacted water bodies. These include pharmaceuticals, steroid hormones, pesticides and industrial chemicals. The resistance of certain TrOC to conventional wastewater treatment and their potential adverse effects on human and ecological health raise significant concerns (Snyder, 2008). This has prompted research on TrOC degradation by white-rot fungi (Yang et al., 2013b).

White-rot fungi can efficiently degrade a wide range of organic compounds including TrOC that are resistant to bacterial degradation via one or more extracellular enzymes including lignin peroxidases, manganese-dependent peroxidases and laccase. Apart from the extracellular enzymes, intracellular enzyme systems, such as cytochrome P450, have been reported to play important roles in the removal of some TrOC (Hata et al., 2010). In addition to whole-cell preparations, removal of TrOC has been investigated either by employing crude culture extract ('crude enzyme') or by purified enzymes (Marco-Urrea et al., 2009, Zhang and Geißen, 2010). Enzyme-catalysis has been shown to be enhanced by the addition of low molecular weight mediator compounds that act as an 'electron shuttle' between the oxidizing enzyme and target compounds (Kim and Nicell, 2006).

Several studies, as noted above, have investigated the TrOC removal capacity of different fungal species. The removal efficiency has been observed to depend on fungal species, their specific enzyme systems and the TrOC characteristics. However, with a few exceptions (Tran et al., 2010, Rodarte-Morales et al., 2011), individual studies to date have focused mostly on a single or only a few TrOC at a time. This has hindered the establishment of a uniform database on white-rot fungal degradation of a broad spectrum of TrOC, particularly because of the differences in experimental conditions in individual studies. Furthermore, some TrOC categories such as UV filters (active ingredients of sunscreens) and phytoestrogens have not been investigated by the previous studies.

Enzymatic degradation of a range of TrOC was investigated in a previous study (Nguyen et al., 2013). However, it is noteworthy that because of the combined effect of intracellular, mycelium-bound and extracellular enzymes as well as sorption of TrOC on the biomass, whole-cell white-

rot fungal treatment may cover a wider spectrum of TrOC compared with enzymatic treatment. A few studies have separately compared the performance of whole-cell and extracellular extract (Auriol et al., 2007, Tran et al., 2010) or have studied the role of intracellular enzyme systems (Marco-Urrea et al., 2009, Hata et al., 2010). However, systematic studies on the relative contribution of biosorption and various modes of biodegradation (e.g., extracellular enzyme dependent/independent) during fungal removal of TrOC remain scarce. Elucidation of such mechanisms is a prerequisite to formulation of strategies to ensure stable operation of continuous flow fungal reactors (Yang et al., 2013b).

A series of batch tests was conducted in this study to address the research gaps outlined above. The removal of 30 TrOC belonging to diverse groups in terms of applications or origins and chemical structures by a whole-cell pure fungus culture (*Trametes versicolor*) and the fungal extracellular enzyme extract has been systematically compared. In discussing the removal efficiency, the relative contribution of biosorption and biodegradation is addressed, as well as potential catalytic modes (extracellular enzyme dependent/independent) responsible for fungal degradation of TrOC. The effect of augmenting enzymatic transformation with a redox mediator on the removal performance and the treated media toxicity was also studied.

2. Materials and methods

2.1 Trace organic contaminants

A mixture of 30 TrOC, including 11 pharmaceuticals, six pesticides, five steroid hormones, three industrial precursors and products, three UV filters and two phytoestrogens was used in this study. Key properties of these compounds are listed in Supplementary data Table S1. These were selected in view of their widespread occurrence in wastewater and wastewater-impacted water bodies and their diverse physicochemical properties (e.g. hydrophobicity and molecular weight). Analytical grade chemicals were purchased from Sigma-Aldrich (Australia). A mixed stock solution of the TrOC was prepared at a concentration of 1 g L⁻¹ (each) in pure methanol, stored at -18°C and used within one month.

2.2 Fungus culture and preparation of crude enzyme extract

The white-rot fungus *T. versicolor* ATCC 7731 (USA) was used in this study. Malt extract broth (Merck, Germany) was used as the growth and the test medium at concentrations of 5 g L⁻¹ and 1

g L⁻¹, respectively. The pH of the growth medium as well as the batch test medium was adjusted to 4.5 with HCl. Refrigerated stock fungal suspension was grown on a rotary shaker at 28 °C in sterilized growth medium for a week (Yang et al., 2013a). Freshly grown fungal pellets and the supernatant of the fungal culture ('crude enzyme extract') were then harvested and subsequently used for whole-cell and crude enzyme batch tests.

2.3 Batch test protocol

Batch tests were conducted under three broad categories to assess: i) removal by live and 'chemically inactivated' (biosorption only) whole-cell preparations; ii) performance of the live whole-cell in the presence of an intracellular cytochrome P450 inhibitor; iii) degradation by the crude enzyme extract with/without addition of a redox-mediator.

In the enzymatic degradation test, the stock TrOC solution was added to 25 mL crude enzyme extract (See section 2.2) in 100 mL beakers at a final TrOC concentration of 100 µg L⁻¹ (each). The crude enzyme extract demonstrated a laccase activity of 35 µM substrate min⁻¹ (See Section 2.4.2). Additional beakers were incubated to assess the effect of addition of the redox mediator 1- hydroxybenzotriazole, (HBT, 1 mM) (Tran et al., 2010). Control samples comprised TrOC in deionised (Milli-Q) water. The beakers were covered with aluminium foil and incubated in a rotary shaker at 70 rpm and 25 °C for 24 h. The experiments were conducted in triplicate. At the end of the incubation period, the whole test medium was harvested. The samples were diluted to 500 mL with deionised (Milli-Q) water and filtered through 0.45 µm glass fibre filter (Filtech, Australia). The pH of the sample was adjusted to 2 by using 4 M H₂SO₄ before refrigerating until solid-phase extraction (for GC/MS analysis, see Section 2.4.1) within 2 d. For toxicity testing, undiluted samples were kept at 4 °C until analysis.

During the whole-cell test, approximately 0.4 g L⁻¹ freshly grown fungus culture was inoculated to sterile test medium (50 mL) in 400 mL sterile beakers. TrOC were added to the test solution at final concentrations of 50 µg L⁻¹ (each). Separate beakers were incubated to assess the removal by sodium azide (0.1 mM)-inactivated fungus culture (Yang et al., 2011). The intracellular enzyme inhibition test followed a similar procedure by adding 1-aminobenzotriazole (1mM) (Hata et al., 2010) to inactivate intracellular cytochrome P450 enzyme systems. The containers

were incubated in the same fashion mentioned above. All experiments were conducted in triplicate and a sampling method similar to that in the tests with enzyme solution was adopted.

2.4 Analytical methods

2.4.1 TrOC analysis

The TrOC concentrations in the samples were measured using a Shimadzu GC/MS (QP5000) system following a previously reported method (Hai et al., 2011). The GC/MS system was equipped with a Shimadzu AOC 20i autosampler and a Phenomenex Zebron ZB-5 (5% diphenyl-95% dimethyl polysiloxane) capillary column (30 m x 0.25 mm ID, $d_f = 0.25 \mu\text{m}$). The quantitative detection limits of this analytical method were compound specific and in the range of 1 to 20 ng L⁻¹ (Supplementary data Table S1). Statistical analysis of data was performed using the *t*-test function in Microsoft Excel. Values of $p < 0.05$ were considered to indicate statistical significance.

2.4.2 Enzymatic activity and toxicity assay

Under the experimental conditions of this study, the fungus predominantly secreted the extracellular enzyme laccase. Laccase activity was measured by monitoring the change in absorbance at 468 nm due to the oxidation of 2,6-dimethoxy phenol (DMP) at room temperature over 2 min using a spectrophotometer (PharmaSpec UV-1700, Shimadzu, Kyoto, Japan). Laccase activity was calculated from the molar extinction coefficient $\varepsilon = 49.6 (\text{mM}\cdot\text{cm})^{-1}$ and expressed in μM substrate min⁻¹. The toxicity of the treated solution was tested in duplicate by measuring bioluminescence inhibition in *Photobacterium leiognathi* (ToxScreen3 assay, CheckLight Ltd, Israel) as described previously (Nguyen et al., 2013). Toxicity was expressed as Relative Toxic Unit (rTU) calculated as $\text{rTU} = 1/\text{IC}_{20}$, with IC_{20} (concentration of the sample required to kill 20% of the bacteria) determined by linear regression of the toxicity response vs. relative sample enrichment within the linear range ($\text{IC}_{00} - \text{IC}_{40}$).

3. Results and discussion

3.1 Removal performance of live and chemically inactivated whole-cell

Biodegradation, biosorption, photodegradation and volatilization are the possible removal mechanisms of TrOC (Yang et al., 2013b). Photodegradation of the TrOC was minimized by

covering the test containers (Section 2.3), while the volatilization of the selected compounds was negligible given their low vapor pressure (Supplementary data Table S1). Therefore, biodegradation and biosorption were considered as potential removal mechanisms under the tested conditions.

Hydrophobic interactions play a very important role in sorption of TrOC to biomass. According to a definition widely accepted in the literature, an organic compound is hydrophobic when its pH-dependent effective octanol water partitioning coefficient (measured by log D) is 3 or higher (Hai et al., 2013). In this study, for almost all significantly hydrophobic compounds (log D $\geq$ 4) similar levels (> 50%) of removal were achieved by the live and sodium azide-inactivated (biosorption only) cultures (Figure 1). In particular, over 80% removal of 17 β -estradiol 17-acetate, pentachlorophenol, 4-*tert*-octylphenol and triclosan was achieved by the live and inactivated cultures. No previous study on the removal of 17 β -estradiol 17-acetate by whole-cell white-rot fungi could be found. However, the results in the current study are in good agreement with the significant removal of 4-*tert*-octylphenol (Tamagawa et al., 2007), triclosan (Cajthaml et al., 2009) and pentachlorophenol (Ryu et al., 2000) reported in previous studies. Because a similar removal was attained of these significantly hydrophobic TrOC by both live and inactivated cultures, the governing mode of removal (biodegradation or biosorption) could not be ascertained from their aqueous phase concentration change. Nevertheless further insight into this aspect has been provided in the following section while comparing the performance of whole-cell and crude enzyme extract.

[Figure 1]

Of particular interest was the high (60-99%) removal of five hydrophobic (log D=3-4) compounds, namely, naproxen, formononetin, ibuprofen, estrone and bisphenol A by the live culture, but relatively less removal by the inactivated culture ($p < 0.05$, Supplementary data Table S2). This observation suggests that biodegradation (and not biosorption) was the main mechanism of removal of these compounds. No previous reports regarding the removal of formononetin by white-rot fungi could be identified. However, previous studies have revealed high removal of naproxen (Marco-Urrea et al., 2010, Rodarte-Morales et al., 2010, Tran et al., 2010, Rodarte-Morales et al., 2011), ibuprofen (Marco-Urrea et al., 2009), estrone (Tamagawa et al., 2005) and bisphenol A (Cajthaml et al., 2009, Yang et al., 2013c) by whole-cell white-rot

fungi treatment. The high removal of these TrOC is also in good agreement with a TrOC classification proposed by Yang et al. (2013b) based on their degradation by white-rot fungi.

As expected, 12 hydrophilic compounds possessing a $\log D < 3$ showed negligible removal by the inactivated culture, confirming their negligible biosorption (Figure 1). However, their removal by the live culture was also generally low or negligible, indicating their resistance to degradation by the *T. versicolor* strain used in this study. These hydrophilic TrOC include some highly resistant compounds (showing low or variable removal) reported in the literature (Yang et al., 2013b). For instance, carbamazepine has been shown to be resistant to fungal treatment by whole-cell culture (Marco-Urrea et al., 2009) and enzymatic treatment (Marco-Urrea et al., 2009, Zhang and Geißen, 2010, Eibes et al., 2011), although certain species were able to degrade it well (Rodarte-Morales et al., 2010, Rodarte-Morales et al., 2011). Marco-Urrea et al. (2009) observed 97% removal of clofibric acid by a different strain of *T. versicolor* than used in this study, however, lower removal (0-24%) by *Irpex lacteus* and *Phanerochaete chrysosporium*. Atrazine was found to be resistant to treatment by *Dichotomitus squalens*, *Phanerochaete velutina*, and *Hypholoma fasciculare* (Bending et al., 2002); however, a different strain of *T. versicolor* was reported to achieve a better removal (Bending et al., 2002). There is further discussion on the behavior of the resistant TrOC in regards to their molecular structures in the next section.

3.2 Comparison of removal by whole-cell and crude enzyme extract

3.2.1 Removal mode

Highly hydrophobic compounds may be removed solely by biosorption. However, when the performance of live/inactivated whole-cell culture (Figure 1) and that of crude enzyme extract (Figure 2) is taken into consideration, it can be confirmed that most of the very hydrophobic TrOC ($\log D > 3$) in this study were in fact biodegraded. Performance comparison between whole-cell and crude enzyme extract also helped identify the TrOC for which low or negligible removal by the latter was observed ($p < 0.05$, Supplementary data Table S2). These include: three very hydrophobic TrOC (oxybenzone, gemfibrozil, and pentachlorophenol), two moderately hydrophobic TrOC (formononetin and ibuprofen), and two significantly hydrophilic TrOC (salicylic acid and naproxen) (Figure 2). Better performance of the whole-cell culture for these

compounds indicated three possibilities: i) biosorption in fact was the main mechanism (i.e, resistant to biodegradation), or, ii) intracellular cytochrome P450 enzyme system was significantly involved in degradation by the whole-cell, or, iii) mycelium-associated enzyme played a significant role in biodegradation.

[Figure 2]

As noted in Section 2.3, the role of intracellular cytochrome P450 was assessed by comparing the removal by whole-cell culture in the presence and absence of 1-aminobenzotriazole (a cytochrome P450 inhibitor). The cytochrome P450 inhibitor was found to have a negligible impact on degradation of all except seven TrOC, namely, salicylic acid, ibuprofen, gemfibrozil, naproxen, bisphenol A, diclofenac and 17 β -estradiol (Figure 3). The role of intracellular enzymes in the fungal degradation of salicylic acid, bisphenol A and 17 β -estradiol has not been studied before. However, the data in the current is consistent with previous studies reporting the negative impact of cytochrome P450 inhibition on the degradation of naproxen (Marco-Urrea et al., 2010), ibuprofen (Marco-Urrea et al., 2009), gemfibrozil (Tran et al., 2010) and diclofenac (Hata et al., 2010). Previous reports have also demonstrated the important role of mycelium-associated enzymatic activity in the degradation of resistant compounds such as dye (Svobodova et al., 2008) and TrOC (Yang et al., 2013a). Considering the data in Figures 1-3 collectively, in this study, the reason for lower removal by the crude enzyme extract compared to that by the whole-cell culture can be considered compound-specific. For salicylic acid, naproxen, formononetin, ibuprofen and gemfibrozil, the role of cytochrome P450 and/or mycelium-associated enzymes was critical, while for oxybenzone and pentachlorophenol the role of biosorption was significant in their removal. Further clarification on this aspect is presented in Section 3.3.

[Figure 3]

3.2.2 Impact of enzymatic activity

It is noted that similar removal by whole-cell and crude enzyme extract was obtained for bisphenol A and diclofenac (Figure 2) although a significant (but not exclusive) role of intracellular enzymes in the degradation of these TrOC was evident (Figure 3). Furthermore, for two hydrophilic compounds, namely, estriol and 4-*tert*-butylphenol, the extracellular enzyme

extract achieved better and/or more stable removal (Figure 2). This was despite the fact that a higher initial concentration was applied during the test with crude enzyme extract ($100\text{ }\mu\text{g L}^{-1}$ of each compound, compared to $50\text{ }\mu\text{g L}^{-1}$ of each in the whole-cell test). This may be attributed to the fact that the laccase activity in the whole-cell culture gradually increased from zero to approximately $20\text{ }\mu\text{M min}^{-1}$ within 24 h, while that in the extracellular extract remained at $35\text{ }\mu\text{M min}^{-1}$ from the start of the test (Figure 4). Auriol et al. (2007) previously observed the removal of synthetic hormones including estriol to depend on the level of laccase activity in the test media. In that study, estriol was completely removed by a laccase solution exhibiting an activity of 20 U mL^{-1} , while the removal corresponding to a laccase activity of 5 U mL^{-1} was 88%. Tran et al. (2010) also reported increased removal of pharmaceuticals such as naproxen (from 30 to 100%), ibuprofen (from 10 to 40%) and ketoprofen (from 25 to 50%) with the increase of laccase activity from 2 to 6 U mL^{-1} . The potential effect of level of laccase activity on the removal of the above TrOC in this study is also indicative of their relatively resistant structure against fungal degradation.

[Figure 4]

3.2.3 Resistant TrOC

Under the conditions tested in this study, little or no (<30%) removal of 15 TrOC, namely, metronidazole, primidone, amitriptyline, propoxur, clofibric acid, carbamazepine, enterolactone, fenoprop, atrazine, ametryn, ketoprofen, benzophenone and octocrylene (Category 3, Figure 2) and estriol and 4-*tert*-butylphenol (Category 2, Figure 2) was achieved by the whole-cell culture. The crude enzyme extract could degrade well estriol and 4-*tert*-butylphenol but no or low degradation of additional six TrOC including salicylic acid, naproxen, formononetin, ibuprofen, gemfibrozil and pentachlorophenol was obtained (Figure 2). This section particularly discusses the case of 13 TrOC for which treatment by neither whole-cell nor crude enzyme preparations was found effective (Category 3, Figure 2). It is noteworthy that the removal of only a few (e.g., clofibric acid, carbamazepine, atrazine and ketoprofen) of these 13 TrOC by whole-cell culture has been studied before; however, the existing literature confirms the resistance of these TrOC to fungal degradation (Yang et al., 2013b).

The resistance of the aforementioned TrOC can be attributed to their chemical structures. The presence of electron withdrawing groups (EWG) generates an electron deficiency and thus

renders the compounds less susceptible to oxidative catabolism (Tadkaew et al., 2011). The low removal of the chlorinated TrOC clofibric acid and fenoprop can be explained by the fact that chlorine is a strong EWG. Treatment by laccase from *T. versicolor* was reported to inefficiently remove clofibric acid (Tran et al., 2010). Atrazine and ametryn are two triazine compounds. Atrazine additionally contains a chloride group. Despite having the weak electron donating groups (EDG) methyl and secondary amine, atrazine and ametryn were poorly removed. This is consistent with the report of Mougin et al. (1997) who found that triazine pesticides are resistant to fungal treatment. The insignificant removal of metronidazole, propoxur and carbamazepine can be attributed to the presence of strong EWG nitro, carbamate and amide groups, respectively in their structures. The presence of these groups has been reported to cause higher resistance to biodegradation (Tadkaew et al., 2011, Yang et al., 2013b). In a previous study, at an initial concentration of 10 $\mu\text{g L}^{-1}$, complete removal of ketoprofen was reported by the whole-cell of a different strain of *T. versicolor*, while only 12% removal was achieved by the extracellular extract (Tran et al., 2010). Notably, ketoprofen contains the carboxylic acid group which is a strong EWG. Its degradation pathway may be limited to being via hydroxylation of the vicinal unsubstituted aromatic fragment and the mono-carbon-substituted benzenoid (Quintana et al., 2005), and this pathway may have been unavailable to the *T. versicolor* strain utilized in this study.

3.3 Effect of mediator addition on removal by crude enzyme extract

Figure 5 shows the effect of addition of the mediator to extracellular extract on the removal of the 30 selected TrOC. Of the TrOC for which negligible degradation was attained by the crude enzyme extract (Figure 2), the addition of HBT significantly improved (from 20 to over 90% improvement) the removal of seven of them including amitriptyline, ametryn, primidone, salicylic acid, enterolactone, atrazine and oxybenzone ($p < 0.05$, Supplementary data Table S2). HBT addition also improved the removal of pentachlorophenol, diclofenac and naproxen by 60, 25 and >75%, respectively, achieving above 90% removal of each. Notable among these were salicylic acid, naproxen, oxybenzone, and pentachlorophenol, which were well-removed by whole-cell (combined biosorption and/or biodegradation via extracellular, intracellular or mycelium-bound enzyme systems) but showed low or no removal by extracellular extract (in

absence of HBT) (Section 3.2.1). The role of HBT in improving the enzymatic degradation of these TrOC is in good agreement with a previous study (Nguyen et al., 2013).

[Figure 5]

The better performance of the mediator-amended crude enzyme solution may be explained by two factors attributable to the aminoxyl radical species generated from HBT by laccase: i) reduction of steric hindrance, and ii) higher redox potential. It is well known that laccase promotes the single electron oxidation of phenols (Yang et al., 2013b). However, steric factors may hinder the approach of the substrate to the active site of the enzyme and, consequently, inhibit the oxidation of even some phenolic substrates. The aminoxyl radicals generated from HBT by laccase, due to their small size, can abstract H-atom from the O–H bond of these phenolic substrates and can form the corresponding phenoxyl radicals (d'Acunzo et al., 2006). These phenoxyl radicals in turn react with the substrate via a radical hydrogen atom transfer route (Coniglio et al., 2008) that improves the biodegradation potential. This mechanism may explain the significantly improved degradation of the phenolic TrOC such as salicylic acid, enterolactone and oxybenzone in the presence of HBT (Figure 5). The laccase-HBT system can also achieve better removal of non-phenolic compounds as the aminoxyl radicals have redox potentials in the order of 1.0–1.1 V (d'Acunzo et al., 2006), which are higher than that of laccase only (0.17V) (Klonowska et al., 2002). For instance, Bernini et al. (2011) reported that the laccase-mediator system can effectively trigger the H-abstraction from benzylic carbons of non-phenolic compounds. This can explain the improvement in atrazine, amitriptyline and ametryn removal in the current study in the presence of HBT. Aminoxyl radicals generated from oxidation of HBT by laccase from the fungus *Trametes villosa* were previously reported to carry out H-abstraction from certain amide substrates (Coniglio et al., 2008). However, no improvement in the removal of the amide compound carbamazepine in this study is probably due to the difference in redox potential of laccase from different *Trametes* species as well as the specific structures of the compounds.

[Table 1]

3.4 Effect of treatment mode on effluent toxicity

While TrOC transformation via fungal treatment may often lead to detoxification (Jelic et al., 2012), increase in toxicity of white-rot fungi-treated media containing TrOC has also been reported. For example, Kim and Nicell (2006) reported that the addition of the mediator 2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid enhanced the laccase catalysed oxidation of aqueous triclosan, but negatively affected the residual toxicity. A recent study has reported increased effluent phytotoxicity following laccase-HBT catalysed treatment of a dye solution (Daassi et al., 2013). However, there appears to be no specific literature regarding toxicity following laccase-HBT catalysed treatment of TrOC. In this study, a ToxScreen3 assay (Section 2.4.2) revealed a slight decrease in toxicity after treatment by whole-cell or crude enzyme extract. For instance, the toxicity of the TrOC solution in Milli-Q water was 14.7 ± 1.0 rTU, which reduced to 10.7 ± 1.1 rTU after the treatment by crude enzyme extract. In comparison, there was a significant increase in toxicity (88.6 ± 3.9 rTU) after the addition of HBT (1 mM). Further investigations such as identification of metabolites (whole-cell culture, extracellular extract and purified laccase with and without redox mediators) are needed to clarify the relative contribution of TrOC-metabolites and HBT to treated media toxicity.

3.5 Insight into overall removal of TrOC classes

Generalizations regarding TrOC removal based on the intended applications or origins can be difficult as compounds within the same TrOC class can have vastly different chemical structures (Hai et al., 2013). Nevertheless, some interesting trends regarding fungal degradation of different TrOC classes can be observed in this study (Table 1). In line with several previous studies (Tamagawa et al., 2005, Cajthaml et al., 2009, Yang et al., 2013b) all hormones and the industrial chemicals were well degraded. Among these, the degradation of estriol (which is a hormone) and 4-*tert*-butylphenol (which is an industrial chemical) appeared to be more sensitive to the level of enzymatic activity (Section 3.2.2).

The removal of the pharmaceutically active compounds by whole-cell culture varied over a wide range: no removal (metronidazole, amitriptyline, primidone, carbamazepine and ketoprofen) to over 80% removal (salicylic acid, naproxen, ibuprofen, gemfibrozil, diclofenac and triclosan).

The removal of salicylic acid, primidone and naproxen by crude enzyme extract was significantly improved by HBT addition.

In good agreement with the literature (Mougin et al., 1997, Tran et al., 2010, Yang et al., 2013b), pesticides demonstrated particular resistance to degradation. Among the six pesticides, no degradation was observed for clofibric acid, propoxur and fenoprop. HBT addition was essential for the degradation of ametryn (30%), atrazine (>90%) and pentachlorophenol (>90%).

Degradation of UV filters and phytoestrogens by white-rot fungi has not been studied before. In this study, among the UV filters, high removal of oxybenzone was achieved by the live whole-cell culture, but degradation by the crude enzyme required HBT addition. Benzophenone and octocrylene were removed only by the live whole-cell culture. Of the two phytoestrogens, formononetin was degraded only by whole-cell preparations, while enterolactone degradation was only possible by amending the extracellular extract with HBT.

Comparing the performance of the fungal strain used in this study with that of conventional bacterial activated sludge was not the aim of this study. However, it is worth noting that the results in the current study suggest differences in the mode of removal and the spectrum of TrOC covered by these options. The white-rot fungi strain achieved significant degradation of some pharmaceuticals such as naproxen and diclofenac, and the pesticide atrazine, which are usually reported to be highly resistant to bacterial degradation (Hai et al., 2013). It is furthermore noteworthy that the significantly hydrophobic TrOC such as many of the hormones, personal care products and alkyl phenolic industrial compounds are well removed from the aqueous phase during conventional (bacterial) wastewater treatment. However, some of these TrOC such as 17 β -estradiol, 4-*tert*-octyl phenol and triclosan exhibit significant resistance to bacterial degradation (Hai et al., 2013). The high biosorption of these resistant TrOC in conventional treatment poses the risk of leaching of these compounds during sludge processing and disposal. By contrast, the data reported in this study confirm fungal degradation of these TrOC (at least the disappearance of the parent molecules).

4. Conclusion

In this study, all 14 phenolic TrOC but only a half of the tested non-phenolic TrOC were effectively degraded. All hydrophobic (log D>3) TrOC were well removed. By comparing the

performance of the whole-cell preparations and the extracellular extract (no biosorption), it was confirmed that biodegradation was the main mechanism of removal. However, the fact that the hydrophilic compounds were poorly removed may indicate the importance of biosorption in subsequent degradation by the whole-cell. In addition, the lower degradation of some hydrophobic compounds by the extracellular extract, and the impact of intracellular cytochrome P450 system inhibition on the degradation of some TrOC by the whole-cell culture indicated the importance of extracellular enzyme-independent catalytic pathways. Addition of the mediator 1-hydroxy benzotriazole to the crude enzyme extract led to improvement of some phenolic as well as non-phenolic TrOC degradation. However, this was accompanied by higher residual toxicity in the treated media. The degradation profile of the tested TrOC by the fungal culture is quite different from that by activated sludge processes.

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References

- Auriol, M., Filali-Meknassi, Y., Tyagi, R. D. & Adams, C. D. 2007. Laccase-catalyzed conversion of natural and synthetic hormones from a municipal wastewater. *Water Research*, 41, 3281-3288.
- Bending, G. D., Friloux, M. & Walker, A. 2002. Degradation of contrasting pesticides by white rot fungi and its relationship with ligninolytic potential. *FEMS Microbiology Letters*, 212, 59-63.
- Bernini, R., Crisante, F., Gentili, P., Morana, F., Pierini, M. & Piras, M. 2011. Chemoselective C-4 Aerobic Oxidation of Catechin Derivatives Catalyzed by the *Trametes villosa* Laccase/1-Hydroxybenzotriazole System: Synthetic and Mechanistic Aspects. *The Journal of Organic Chemistry*, 76, 820-832.
- Cajthaml, T., Křesinová, Z., Svobodová, K. & Möder, M. 2009. Biodegradation of endocrine-disrupting compounds and suppression of estrogenic activity by ligninolytic fungi. *Chemosphere*, 75, 745-750.
- Coniglio, A., Galli, C., Gentili, P. & Vadalà, R. 2008. Oxidation of amides by laccase-generated aminoxyl radicals. *Journal of Molecular Catalysis B: Enzymatic*, 50, 40-49.
- d'Acunzo, F., Galli, C., Gentili, P. & Sergi, F. 2006. Mechanistic and steric issues in the oxidation of phenolic and non-phenolic compounds by laccase or laccase-mediator systems. The case of bifunctional substrates. *New Journal of Chemistry*, 30, 583-591.

- Daassi, D., Zouari-Mechichi, H., Frikha, F., Martinez, M., Nasri, M. & Mechichi, T. 2013. Decolorization of the azo dye Acid Orange 51 by laccase produced in solid culture of a newly isolated *Trametes trogii* strain. *3 Biotechnology*, 3, 115-125.
- Eibes, G., Debernardi, G., Feijoo, G., Moreira, M. & Lema, J. 2011. Oxidation of pharmaceutically active compounds by a ligninolytic fungal peroxidase. *Biodegradation*, 22, 539-550.
- Hai, F. I., Tessmer, K., Nguyen, L. N., Kang, J., Price, W. E. & Nghiem, L. D. 2011. Removal of micropollutants by membrane bioreactor under temperature variation. *Journal of Membrane Science*, 383, 144-151.
- Hai, F. I., Nghiem, L. D., Khan, S. J., Price, W. E. & Yamamoto, K. 2013. Wastewater reuse: Removal of emerging trace organic contaminants. In: Hai, F. I., Yamamoto, K. & Lee, C.-H. (eds.) *Membrane Biological Reactors*. London (ISBN: 9781780400655): IWA publishing, pp. 163-203.
- Hata, T., Kawai, S., Okamura, H. & Nishida, T. 2010. Removal of diclofenac and mefenamic acid by the white rot fungus *Phanerochaete sordida* YK-624 and identification of their metabolites after fungal transformation. *Biodegradation*, 21, 681-9.
- 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 Research*, 46, 955-964.
- Kim, Y. J. & Nicell, J. A. 2006. Laccase catalysed oxidation of aqueous triclosan. *Journal of Chemical Technology and Biotechnology*, 81, 1344-1352.
- Klonowska, A., Gaudin, C., Fournel, A., Asso, M., Le Petit, J., Giorgi, M. & Tron, T. 2002. Characterization of a low redox potential laccase from the basidiomycete C30. *European Journal of Biochemistry*, 269, 6119-6125.
- 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.
- Marco-Urrea, E., Pérez-Trujillo, M., Blánquez, P., Vicent, T. & Caminal, G. 2010. Biodegradation of the analgesic naproxen by *Trametes versicolor* and identification of intermediates using HPLC-DAD-MS and NMR. *Bioresource Technology*, 101, 2159-2166.
- Mougin, C., Laugero, C., Asther, M. & Chaplain, V. 1997. Biotransformation of s-Triazine Herbicides and Related Degradation Products in Liquid Cultures by the White Rot Fungus *Phanerochaete chrysosporium*. *Pesticide Science*, 49, 169-177.
- 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. *Bioresource Technology*, 143, 234-241.

- Quintana, J. B., Weiss, S. & Reemtsma, T. 2005. Pathways and metabolites of microbial degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by a membrane bioreactor. *Water Research*, 39, 2654-2664.
- Rodarte-Morales, A., Feijoo, G., Moreira, M. & Lema, J. 2011. Degradation of selected pharmaceutical and personal care products (PPCPs) by white-rot fungi. *World Journal Microbiology and Biotechnology*, 27, 1839-1846.
- Rodarte-Morales, A. I., Moreira, M. T., Feijoo, G. & Lema, J. M. 2010. Evaluation of two fungal strains for the degradation of pharmaceutical and personal care products (PPCPs). *Chemical Engineering Transactions*, 20, 31-36.
- Ryu, W. R., Shim, S. H., Jang, M. Y., Jeon, Y. J., Oh, K. K. & Cho, M. H. 2000. Biodegradation of pentachlorophenol by white rot fungi under ligninolytic and nonligninolytic conditions. *Biotechnology and Bioprocess Engineering*, 5, 211-214.
- Snyder, S. A. 2008. Occurrence, Treatment, and Toxicological Relevance of EDCs and Pharmaceuticals in Water. *Ozone Science and Engineering*, 30, 65-69.
- Svobodova, K., Majcherczyk, A., Novotny, C. & Kues, U. 2008. Implication of mycelium-associated laccase from *Irpex lacteus* in the decolorization of synthetic dyes. *Bioresource Technology*, 99, 463-471.
- Tadkaew, N., Hai, F. I., McDonald, J. A., Khan, S. J. & Nghiem, L. D. 2011. Removal of trace organics by MBR treatment: The role of molecular properties. *Water Research*, 45, 2439-2451.
- Tamagawa, Y., Hirai, H., Kawai, S. & Nishida, T. 2005. Removal of estrogenic activity of endocrine-disrupting genistein by ligninolytic enzymes from white rot fungi. *FEMS Microbiology Letters*, 244, 93-98.
- Tamagawa, Y., Hirai, H., Kawai, S. & Nishida, T. 2007. Removal of estrogenic activity of 4-tert-octylphenol by ligninolytic enzymes from white rot fungi. *Environmental Toxicology*, 22, 281-286.
- Tran, N. H., Urase, T. & Kusakabe, O. 2010. Biodegradation characteristics of pharmaceutical substances by whole fungal culture *Trametes versicolor* and its laccase. *Journal of Water and Environmental Technology*, 8, 125-140.
- Yang, S.-F., Lin, C.-F., Yu-Chen Lin, A. & Andy Hong, P.-K. 2011. Sorption and biodegradation of sulfonamide antibiotics by activated sludge: Experimental assessment using batch data obtained under aerobic conditions. *Water Research*, 45, 3389-3397.
- Yang, S., Hai, F. I., Nghiem, L. D., Nguyen, L. N., Roddick, F. & Price, W. E. 2013a. Removal of bisphenol A and diclofenac by a novel fungal membrane bioreactor operated under non-sterile conditions. *International Biodeterioration and Biodegradation*, 85, 483-490.

- Yang, S., Hai, F. I., Nghiem, L. D., Price, W. E., Roddick, F., Moreira, M. T. & Magram, S. F. 2013b. Understanding the factors controlling the removal of trace organic contaminants by white-rot fungi and their lignin modifying enzymes: a critical review. *Bioresource Technology*, 141, 97-108.
- Yang, S., Hai, I. F., Nghiem, D. L., Roddick, F. & Price, W. E. 2013c. Removal of trace organic contaminants by nitrifying activated sludge and whole-cell and crude enzyme extract of *Trametes versicolour*. *Water Science and Technology*, 67, 1216-1223.
- Zhang, Y. & Geißen, S.-U. 2010. In vitro degradation of carbamazepine and diclofenac by crude lignin peroxidase. *Journal of Hazardous Material*, 176, 1089-1092.

Table 1: Comparative removal of TrOC classes by different treatment options.

Category	Compound	Phenolic moiety	Log <i>D</i> (pH 4)	Removal (%) by different options			
				I	II	III	IV
Pharmaceuticals	Metronidazole		-0.2	0	0	17 ±23	10 ±15
	Salicylic acid	Yes	0.2	0		0	
	Primidone		0.8	24 ±34	10 ±7	0	
	Amitriptyline		1.3	8 ±8	5 ±4	4±1	
	Carbamazepine		1.9	6±5	0	4 ±5	0
	Ketoprofen		2.7	10 ±15	24 ±8	0	7 ±5
	Naproxen		2.8	14±5		20 ±3	
	Ibuprofen		3.4	32 ±1		2 ±2	0
	Gemfibrozil		4.2			18 ±4	24 ±3
	Diclofenac		4.3				
	Triclosan	Yes	5.3				
Pesticides	Propoxur		1.5	0	10 ±5	7 ±1	0
	Fenoprop		2.4	0	0	9 ±7	1 ±1
	Clofibric acid		1.6	15 ±3	4 ±5	8 ±2	7 ±8
	Atrazine		2.6	9 ±1	16±15	3 ±2	
	Ametryn		2.6	14 ±8	5 ±6	7 ±7	29 ±1
	Pentachlorophenol	Yes	5.0			10 ±11	
Industrial products	4-tert-Butylphenol	Yes	3.4	10 ±14			
	Bisphenol A	Yes	3.6	26 ±5			
	4-tert-Octolphenol	Yes	5.2				
Steroid hormones	Estriol	Yes	2.5	13 ±4	5 ±2		
	Estrone	Yes	3.6				
	17 α -Ethinylestradiol	Yes	4.1				
	17 β -Estradiol	Yes	4.2				
	17 β -Estradiol-17-acetate	Yes	5.2				
Phytoestrogens	Enterolactone	Yes	1.9	3 ±1	0	19 ±27	
	Formononetin	Yes	2.9	15 ±5		0	0
UV filters	Benzophenone		3.2	10 ±2		0	0
	Oxybenzone	Yes	4.0			4 ±5	
	Octocrylene		6.9	27 ±2		18 ±14	13 ±5

Treatment options: ^IWhole- cell (inactivated), ^{II}Whole-cell (live), ^{III}Crude enzyme extract, and ^{IV}Crude enzyme extract with mediator.

Legend 0 ~ 30% >60%

LIST OF FIGURES

Figure 1 Removal of 30 TrOC from aqueous phase after 24 h of incubation of live and inactivated white-rot fungus in malt extract broth. The initial pH, fungus concentration and the concentration of each of the TrOC were 4.5, 0.4 g L⁻¹ and 50 µg L⁻¹, respectively. Error bars indicate average ± standard deviation (n=3). Fungus was inactivated by adding 0.1 mM sodium azide to the test solution.

Figure 2 Removal of 30 TrOC from aqueous phase after 24 h of incubation of live fungus and crude enzyme extract. Live fungus was incubated in malt extract broth (1 g L⁻¹), and the initial pH, fungus concentration and the concentration of each of the TrOC were 4.5, 0.4 g L⁻¹ and 50 µg L⁻¹, respectively. The initial laccase activity of the crude enzyme extract was 35 µM min⁻¹. TrOC were added to the crude enzyme extract at a concentration of 100 µg L⁻¹. Error bars indicate average ± standard deviation (n=3).

Figure 3 Removal of seven affected TrOC in presence and absence of an intracellular cytochrome P450 inhibitor (1-aminobenzotriazole) at a concentration of 1mM. Error bars indicate average ± standard deviation (n=3).

Figure 4 Level of laccase activity in the media during the test with the whole-cell culture and the crude enzyme extract. Error bars indicate average ± standard deviation (n=3).

Figure 5 Comparative degradation of 30 TrOC by crude enzyme extract with and without a mediator (1-hydroxybenzotriazole, 1mM). Error bars indicate average ± standard deviation (n=3).

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Removal of pharmaceuticals, steroid hormones, phytoestrogens, UV-filters, industrial chemicals and pesticides by *Trametes versicolor*: Role of biosorption and biodegradation

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Table S1: Physicochemical properties of the selected trace organic contaminants (TrOC).

Category	Compound (CAS number)	Molecular weight (g mol ⁻¹)	Log D (pH 4) ^a	Dissociation constant (pKa) ^a	Vapor pressure (mm Hg), at 25°C ^a	Limit of detection (ng L ⁻¹) ^b	Chemical structure
Pharmaceuticals	Ibuprofen (C ₁₃ H ₁₈ O ₂) (5687-27-1)	206.28	2.81	4.41 ± 0.10	1.39E-4	20
	Naproxen (C ₁₄ H ₁₄ O ₃) (22204-53-1)	230.26	2.49	4.84 ± 0.30	3.01E-7	1
	Ketoprofen (C ₁₆ H ₁₄ O ₃) (22071-15-4)	254.28	2.07	4.23 ± 0.10	3.32E-8	20
	Diclofenac (C ₁₄ H ₁₁ Cl ₂ NO ₂) (15307-86-5)	296.15	3.66	4.18 ± 0.10 -2.26 ± 0.50	1.59E-7	5
	Primidone (C ₁₂ H ₁₄ N ₂ O ₂) (125-33-7)	218.25	0.83	12.26 ± 0.40 -1.07 ± 0.40	6.08E-11	10
	Carbamazepine (C ₁₅ H ₁₂ N ₂ O) (298-46-4)	236.27	1.89	13.94 ± 0.20 -0.49 ± 0.20	5.78E-7	10
	Salicylic acid (C ₇ H ₆ O ₃) (69-72-7)	138.12	-0.65	3.01 ± 0.10	4.45E-5	1

Pesticides	Metronidazole (C ₆ H ₉ N ₃ O ₃) (443-48-1)	171.15	-0.14	14.44 ± 0.10 2.58 ± 0.34	2.67E-7	20
	Gemfibrozil (C ₁₅ H ₂₂ O ₃) (25812-30-0)	250.33	3.86	4.75	6.13E-7	1
	Triclosan (C ₁₂ H ₇ Cl ₃ O ₂) (3380-34-5)	289.54	5.34	7.80 ± 0.35	3.36E-5	1
	Amitriptyline C ₂₀ H ₂₃ N (50-48-6)	277.40	2.28	9.18±0.28	1.50E-6	1
	Fenoprop (C ₉ H ₇ Cl ₃ O ₃) (93-72-1)	269.51	1.39	2.93	2.13E-6	20
	Pentachloro-phenol (C ₆ HCl ₅ O) (87-86-5)	266.34	4.62	4.68 ± 0.33	3.49E-4	1
	Atrazine (C ₈ H ₁₄ ClN ₅) (1912-24-9)	215.68	2.63	2.27±0.10	1.27E-5	10
	Propoxur (C ₁₁ H ₁₅ NO ₃) (114-26-1)	209.24	1.54	12.28±0.46 1.49±0.70	1.53E-3	1

	Ametryn (C ₉ H ₁₇ N ₅ S) (843-12-8)	227.33	2.92	3.71±0.41	1.72E-6	10	
	Clofibric acid (C ₁₀ H ₁₁ ClO ₃) (882-09-7)	214.65	0.61	3.18 ±0.10	1.03E-4	1	
Industrial chemicals and their metabolites	4-tert-butylphenol (C ₁₀ H ₁₄ O) (98-54-4)	150.22	3.40	10.13 ± 0.13	0.0361	1	
	4-tert-octylphenol (C ₁₄ H ₂₂ O) (140-66-9)	206.32	5.18	10.15 ± 0.15	1.98E-3	1	
	Bisphenol A (C ₁₅ H ₁₆ O ₂) (80-05-7)	228.29	3.64	10.29 ± 0.10	5.34E-7	1	
Steroid hormones	Estrone (C ₁₈ H ₂₂ O ₂) (53-16-7)	270.37	3.62	10.25 ± 0.40	1.54E-8	5	
	17β-estradiol (C ₁₈ H ₂₄ O ₂) (50-28-2)	272.38	4.15	10.27	9.82E-9	5	

	17 β -estradiol –17 acetate (C ₂₀ H ₂₆ O ₃) (1743-60-8)	314.42	5.11	10.26 $\pm$ 0.60	9.88E-9	5
	17 α ethinylestradiol (C ₂₀ H ₂₄ O ₂) (57-63-6)	269.40	4.11	10.24 $\pm$ 0.60	3.74E-9	10
	Estriol (E3) (C ₁₈ H ₂₄ O ₃) (50-27-1)	288.38	2.53	10.25 $\pm$ 0.70	1.34E-9	10
UV filters	Benzophenone C ₁₃ H ₁₀ O (119-61-9)	182.22	3.21	-	8.23E-4	10
	Oxybenzone C ₁₄ H ₁₂ O ₃ (131-57-7)	228.24	3.89	7.56 $\pm$ 0.35	5.26E-6	10
	Octocrylene C ₂₄ H ₂₇ N O ₂ (6197-30-4)	361.48	6.89	-	2.56E-9	10
	Formononetin C ₁₆ H ₁₂ O ₄ (485-72-3)	268.26	2.86	6.99 $\pm$ 0.20	8.17E-10	5

Phytoestrogens	Enterolactone C ₁₈ H ₁₈ O ₄ (78473-71-9)	298.33	1.89	9.93± 0.10	3.29E-13	5
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^a Source: SciFinder database <https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf>

Log *D* is logarithm of the distribution coefficient which is the ratio of the sum of concentrations of all forms of the compound (ionised and unionised) in octanol and water at a given pH.

na: data not available

^bLimit of detection (LOD) of the compounds during GC-MS analysis as described in Section 2.4. LOD is defined as the concentration of an analyte giving a signal to noise (S/N) ratio greater than 3. The limit of reporting was determined using an S/N ration of greater than 10.

Table S2: Statistical analysis of data.

Compounds	<i>p</i> value		
	Live whole cell vs. inactivated whole cell fungus	Live whole cell vs. crude enzyme extract	Crude enzyme extract vs. mediator-enhanced crude enzyme extract
Metronidazole	-	0.225	0.225
Salicylic acid	0.008	0	0
Primidone	0.489	0.084	0.001
Amitriptyline	0.57	0.75	0.002
Propoxur	0.038	0.141	0.001
Clofibric acid	0.02	0.588	0.037
Carbamazepine	0.122	0.225	0.225
Enterolactone	0.005	0.225	0.018
Fenoprop	-	0.123	0.111
Estriol	0.014	0	0.094
Atrazine	0.333	0.194	0.001
Ametryn	0.255	0.711	0.024
Ketoprofen	0.329	0.022	0.089
Naproxen	0	0	0
Formononetin	0	0.003	-
Benzophenone	0.068	0.048	-
Ibuprofen	0	0	0.225
4-tert-Butylphenol	0.234	0.028	0.009
Estrone	0.004	0.008	0.006
Bisphenol A	0	0.006	0.004
Oxybenzone	0.043	0.002	0.01

17 α -Ethinylestradiol	0.003	0.01	0.056
17 β -Estradiol	0.004	0	0.03
Gemfibrozil	0.001	0.001	0.011
Diclofenac	0.002	0.001	0.021
Pentachlorophenol	0.029	0.021	0.007
17 β -Estradiol-17-acetate	0.022	0	0.014
4-tert-Octylphenol	0.052	0.124	0.418
Triclosan	0	0.001	0.111
Octocrylene	0.173	0.223	0.533