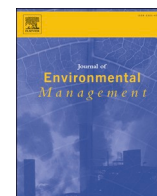


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Research article

Use of lignocellulosic substrate colonized by oyster mushroom (*Pleurotus ostreatus*) for removal of organic micropollutants from waterM. Hultberg^{a,*}, L. Ahrens^b, O. Golovko^b^a Department of Biosystems and Technology, Swedish University of Agricultural Sciences, P.O. Box 103, SE 230 53, Alnarp, Sweden^b Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, P.O. Box 7050, SE 75007, Uppsala, Sweden

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ABSTRACT

Efficient removal techniques are urgently needed to remove organic micropollutants (OMPs) from wastewater, in order to protect water resources. In this study, laccase activity of mushroom substrate colonized by *Pleurotus ostreatus* was evaluated as a novel wastewater treatment method for removal of OMPs, including diclofenac, bicalutamide, lamotrigine, and metformin at environmentally relevant concentrations. Laccase activity of the colonized mushroom substrate was found to be highest, 0.8 enzyme activity (U)/g mushroom substrate wet weight, immediately before initiation of fruiting body formation. The selected OMPs were treated for 5 min with suspensions of mushroom substrate with laccase activity of approximately 50 U/L. Removal of all OMPs was significant, with the highest removal for diclofenac of 90% compared with a control with uncolonized mushroom substrate. To our knowledge, direct use of colonized mushroom substrate in removing diclofenac from water has not been reported previously. Removal efficiency of bicalutamide, lamotrigine, and metformin was 43%, 73%, and 59%, respectively. This demonstrates potential for using mushroom substrate colonized by *P. ostreatus* for removal of OMPs from wastewater.

1. Introduction

Organic micropollutants (OMPs) such as pharmaceuticals and personal care products can be detected in treated wastewater, due to incomplete removal by conventional wastewater treatment technologies (Ba et al., 2018). The OMP concentrations in wastewater generally range from ng to µg per liter (Furlong et al., 2017). Pharmaceuticals are designed to be biologically active and may affect non-target organisms, causing adverse effects (Brodin et al., 2013). Thus, to protect water resources, wastewater has to be adequately treated. Wastewater treatment technologies such as ozonation, Fenton oxidation, photocatalytic oxidation, and absorption processes using active carbon are effective in removing OMPs from wastewater (Luo et al., 2014). However, it is often not economically feasible to implement these advanced treatment technologies, and therefore alternative cost-efficient treatment options for wastewater are needed.

Alternative treatment options for wastewater can involve applying oxidative enzymes, including pure and immobilized enzymes (Arca-Ramos et al., 2016) or submerged cultivation of fungi producing oxidative enzymes (Lucas et al., 2016; Mir-Tutusaus et al., 2017; Castellet-Rovira

et al., 2018). The enzymes of interest include different types of peroxidases, such as lignin peroxidase and manganese peroxidase, and phenol oxidases such as laccases. Formation of hydrogen peroxide and free radicals has been demonstrated to be highly important during the degradation process (Munk et al., 2017). The subsequent low substrate specificity of the enzymes, which allows them to act upon a wide range of complex molecules, makes them interesting for bioremediation purposes (Rhodes, 2014). Fungal growth and associated degradation of lignocellulosic waste is a naturally occurring process of fundamental importance for ecosystems (Sánchez, 2010). This process is also increasingly being used for commercial production of edible white-rot fungi, such as oyster mushroom (*Pleurotus ostreatus*), using lignocellulosic-based waste such as sawdust or straw as the substrate (Royse et al., 2017).

Soil amendment with SMS has been shown to be an effective method for degradation of OMPs in soil (Marin-Benito et al., 2016). However, only a few studies have examined direct use of SMS in treating contaminated wastewater. In those studies, efficient degradation of both endocrine disruptors (Kresinova et al., 2018) and chlorothanil (Córdova Juárez et al., 2011) in wastewater using SMS from production of oyster

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mushrooms has been demonstrated. In addition, dyes in contaminated water have been successfully removed using SMS from oyster mushroom production (Papinutti and Forchiassin, 2010). Considering economic feasibility and the need to develop cost-efficient technologies, an interesting finding is that the purity of enzymes used in wastewater treatment is less important (Arca-Ramos et al., 2018). Furthermore, a less pure suspension of enzymes may contain natural mediators, such as small phenolic compounds, which may increase the efficiency of degradation. Thus, direct use of colonized mushroom substrate is a promising method of employing oxidative enzymes for removal of OMPs from wastewater.

The aim of this study was to assess removal of selected OMPs from water using *P. ostreatus* mushroom substrate and relate this to the laccase activity of the colonized substrate. The mushroom substrate consisted of sawdust and wheat bran, and was colonized by a commercial strain of oyster mushroom (*P. ostreatus* M2191). This species was selected as it is easily cultivated on a wide array of substrates and is one of the most widely produced mushrooms in the world (Royse et al., 2017). *P. ostreatus* produces several lignin-degrading enzymes, but with a clear dominance of laccases when grown on lignocellulosic substrate (Fernández-Fueyo et al., 2016). The OMPs were selected based on their annual usage in a wide range of household products and their high occurrence in wastewater and surface waters (rivers and lakes) (Luo et al., 2014). The anti-inflammatory drug diclofenac was used to determine a suitable concentration of colonized mushroom substrate, while the anticancer drug bicalutamide, the antiepileptic drug lamotrigine, and the antidiabetic drug metformin were tested for removal from water. Diclofenac, bicalutamide, lamotrigine and metformin can all be detected in the aquatic environment because of incomplete removal in the wastewater treatment plant, and might have negative impact on aquatic organisms. For example, diclofenac may cause renal lesions and gill alterations in rainbow trout (Triebkorn et al., 2004), or elevate vitellogenin-like proteins in the gonads of female Mediterranean mussels (Gonzalez-Rey and Bebianno, 2014). Another example is that lamotrigine is a hazard due to its neuroactive properties, similarly to other psychoactive pharmaceuticals, which are demonstrated to have an effect on predator-prey relationships among aquatic organisms (Foster et al., 2010).

2. Material and methods

2.1. Chemicals

Diclofenac sodium salt, metformin hydrochloride, lamotrigine, bicalutamide, and isotope-labeled internal standards of diclofenac ($^{13}\text{C}_6$) and carbamazepine (D_{10}) were purchased from Sigma-Aldrich (Sweden). All analytical standards were of a high purity (>98%). Ultrapure water was produced in a Milli-Q Advantage Ultrapure Water purification system and filtered through a 0.22 μm Millipak Express membrane and an LC-Pak[®] polishing unit (Merk Millipore, Billerica, MA). Methanol, acetonitrile, and ammonium acetate, all of high-performance liquid chromatography (HPLC)-grade, were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Mushroom substrate, fruiting body formation, and enzyme production

The fungal species *P. ostreatus* strain M2191 (Mycelia BVBA, Belgium) was used and inoculum was produced as described in Hultberg et al. (2018). A mushroom substrate based on sawdust of alder (73% of dry weight (dwt)), wheat bran (25% of dwt), and calcium sulfate (2% of dwt), and with a moisture content of 65%, was used in the experiments. The mushroom cultivation was performed as described in Hultberg et al. (2018) with an inoculum concentration of 10% (dwt/dwt). Fresh weight, and dry weight after lyophilization of harvested mushrooms, were determined. Mushroom yield (fresh weight) was related to the

amount of substrate (dwt), in order to determine the biological efficiency of the substrate. Total amount of protein in the fruiting bodies was analyzed by the Dumas method (Bellomonte et al., 1987), using a Thermo Scientific[™] FLASH 2000 CHNS/O Analyzer and applying a conversion factor of 4.38 for total nitrogen (Barros et al., 2008).

Samples for analysis of enzyme activity in the mushroom substrate were taken between day 14 and day 36. This time interval covered the period from total colonization of the substrate to primordia formation, harvest of the fruiting bodies in the first flush, and post-harvest. As the mycelium covering the colonized mushroom substrate was hydrophobic, owing to assembly of hydrophobins at the hyphal surface (Asgeirsdóttir et al., 1998), the samples were briefly homogenized (15 s) in a Waring blender at low speed before analyzing enzyme activity as described below.

2.3. Experimental design

The colonized mushroom substrate was briefly homogenized as described above and diluted in 50 mM phosphate buffer at pH 7.0. Controls without mushroom substrate and with uncolonized mushroom substrate (substrate composed as described above but without inoculation of *P. ostreatus*) were included in all experiments. In the first experiment, heat-treated colonized mushroom substrate was also included as a control. Laccase activity was observed at high temperature (70 °C), and thus heat treatment was performed by autoclaving. In the first experiment, treatments with filtered (nylon filter, 1 mm mesh size) substrate solution and unfiltered substrate solution were included, in order to examine the effect of larger particles. No difference between filtered and unfiltered substrate solution was observed, so the main experiments were performed using filtered substrate solution. The laccase activity was analyzed as described above in all treatments.

The OMPs were added after filtration and determination of laccase activity in the suspension. The selected OMPs (diclofenac, bicalutamide, lamotrigine, and metformin) were tested individually in triplicates. The first experiment was performed using diclofenac in a concentration of 2 $\mu\text{g/L}$ and a substrate concentration of 200 g/L wet weight, corresponding to 94 ± 4.3 U/L. The second experiment was performed using diclofenac in concentrations of 2 $\mu\text{g/L}$ and 20 $\mu\text{g/L}$. The substrate concentration was 200, 100, and 50 g/L wet weight, with corresponding laccase activity 79 ± 2.9 , 47 ± 2.3 , and 29 ± 1.0 U/L, respectively. In the third experiment, bicalutamide, lamotrigine, and metformin were tested individually and added to each replicate to reach a concentration of 1–2 $\mu\text{g/L}$ for bicalutamide and lamotrigine and 60 $\mu\text{g/L}$ for metformin. A substrate solution with laccase activity 58 ± 1.0 U/L was used. All treatments were subject to 5 min of incubation on an orbital shaker (100 rpm). The samples were then immediately stored in the freezer at -20 °C before analysis, which was performed within two weeks.

2.4. Enzyme analysis

Laccase activity was determined colorimetrically by detecting the product of oxidation 2,6-dimethoxyphenol (DMP) as described by Parenti et al. (2013). The reaction mixture contained 0.45 mL of diluted sample and 0.5 mL of 10 mM DMP in 100 mM acetate buffer (pH 5). The absorbance was measured at 468 nm, and one unit of enzyme activity (U) was defined as formation of 1 μmol of product per min.

2.5. Chemical analysis

For chemical analysis, the samples were filtered using a regenerated cellulose syringe filter (0.22 mm pores) and 1 mL of the filtered extract was placed in an autosampler vial with 10 ng absolute of the internal standard mixture. The samples were analyzed using a DIONEX UltiMate 3000 ultra-performance liquid chromatography (UPLC) system coupled to a TSQ QUANTIVA triple quadrupole mass spectrometer (MS/MS) system (both Thermo Scientific, Waltham, MA, USA). An Acquity UPLC

BEH-C18 column (100 mm × 2.1 i.d., 1.7 µm particle size, Waters Corporation, Manchester, UK) was used as analytical column. Data were evaluated using TraceFinder™ 3.3 software (Thermo Fisher). Detailed information about the analytical method can be found in Gago-Ferrero et al. (2017).

2.6. Statistical analysis

The data were statistically evaluated using Minitab 18 for Windows. One-way Anova followed by Tukey's multiple comparison test was employed to test for effects of treatments, and the significance level was set to $p < 0.05$.

3. Results

3.1. Mycelial growth and fruiting body production

The mushroom substrate was completely colonized on day 14, primordia were observed on day 22, and harvest of the first flush was performed on day 27. The fruiting body yield was 160 ± 20 g per box, corresponding to a biological efficiency of $90 \pm 11\%$. The moisture content of the fruiting bodies was $91 \pm 0.8\%$ and the protein content was $25 \pm 2.4\%$ of the dry weight.

3.2. Enzyme activity in mushroom substrate over time

The laccase activity over time, expressed as U/L and with a substrate concentration of 50 g wet weight (wwt) of mushroom substrate per liter, is presented in Fig. 1. Laccase activity significantly decreased during cold storage (10 °C), from 38 ± 8.4 U/L at day 14– 22 ± 3.6 U/L at day 18. The laccase activity was restored to initial levels during incubation in the growing chamber (24 °C), so that at day 22, when initiation of fruiting body formation was observed, laccase activity of 39 ± 2.2 U/L was measured. This corresponds to laccase activity of 0.8 U/g (wwt) of mushroom substrate. After this period, a decrease in laccase activity was measured, with 4.3 ± 1.1 U/L on day 29 as the lowest value, which was two days after harvest of the fruiting bodies. This corresponds to a laccase activity of 0.09 U/g (wwt) mushroom substrate. After day 29, an increase in laccase activity was observed, reaching a maximum value of 20 ± 4.1 U/L on day 35.

3.3. OMPs removal after treatment with mushroom substrate

In the first experiment, with laccase activity 94 ± 4.3 U/L, there was

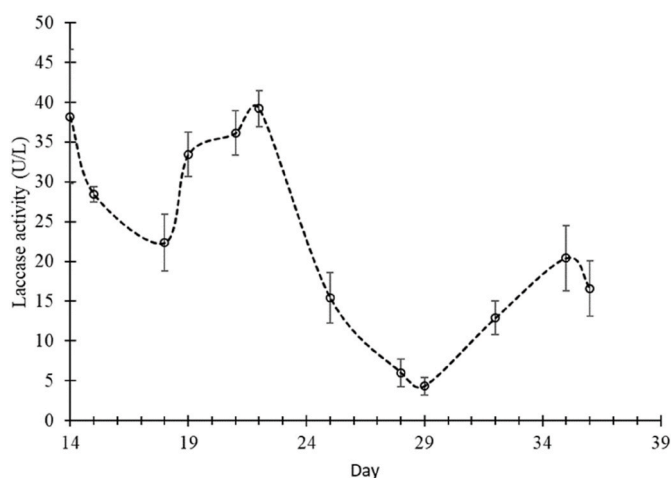


Fig. 1. Laccase activity over time in homogenized suspension of sawdust-based mushroom substrate (50 g/L) colonized by the edible white-rot fungus *Pleurotus ostreatus*. Mean \pm standard deviation are shown, $n = 3$.

a significant decrease in diclofenac concentration in treatments with mushroom substrate (filtered and unfiltered) compared with the controls (Fig. 2). After treatment, the concentration of diclofenac in both the filtered and unfiltered mushroom substrate treatments was 0.3 ± 0.1 µg/L, while that in the control treatment with uncolonized mushroom substrate was 1.8 ± 0.1 µg/L. Thus, use of mushroom substrate colonized by *P. ostreatus* reduced the diclofenac concentration by 85% compared with uncolonized mushroom substrate (Fig. 2). No laccase activity was observed in any of the controls.

In the second experiment, colonized mushroom substrate was used to create suspensions with different levels of laccase activity. A similar concentration of diclofenac (2 µg/L) and range of laccase activity (79 ± 2.9 to 29 ± 1.0 U/L) as in the first experiment were used. The results showed a significant reduction in diclofenac concentration, of 89–96% compared with the control with uncolonized mushroom substrate, in all treatments. A 10-fold higher diclofenac concentration was then treated with the same enzyme suspensions with varying laccase activity (Fig. 3). At the lowest laccase activity (29 U/L), 37% removal of diclofenac was observed, corresponding to an amount of 8.3 µg/L. On increasing the laccase activity to 47 U/L, the removal rate of diclofenac increased significantly, to 92%, corresponding to an amount of 23 µg/L. Increasing the laccase activity further to 79 U/L resulted in diclofenac removal of 97%, which was not a significant increment.

In the third experiment, colonized mushroom substrate was used to create a suspension with laccase activity 58 ± 1.0 U/L, to assess removal of bicalutamide, lamotrigine, and metformin. For all three substances, the treatments with mushroom substrate showed a significant decrease in concentrations compared with the controls (Table 1). Compared with the controls with uncolonized mushroom substrate, the reduction for bicalutamide was 43%, for lamotrigine 73%, and for metformin 59%. In contrast to the experiment with diclofenac, there was a significant difference between the controls, with a significant decrease in concentration observed in the control with uncolonized substrate. This decrease was especially high for metformin, where the uncolonized substrate achieved 80% decrease in concentration compared with the control without substrate. For bicalutamide and lamotrigine, the reduction in concentration achieved by the uncolonized mushroom substrate was 36% and 27%, respectively.

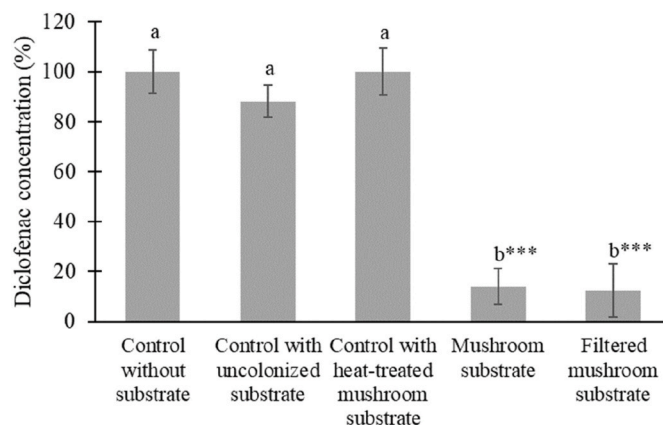


Fig. 2. Residual concentration of diclofenac (%) after 5 min of treatment. Initial diclofenac concentration was 2.0 µg/L and the amount of mushroom substrate used was 200 g/L. Significant difference between the treatments is indicated by different lower-case letters. The level of significance compared to the control is indicated by asterisks, *** $p < 0.001$. Mean \pm standard deviation are shown, $n = 3$.

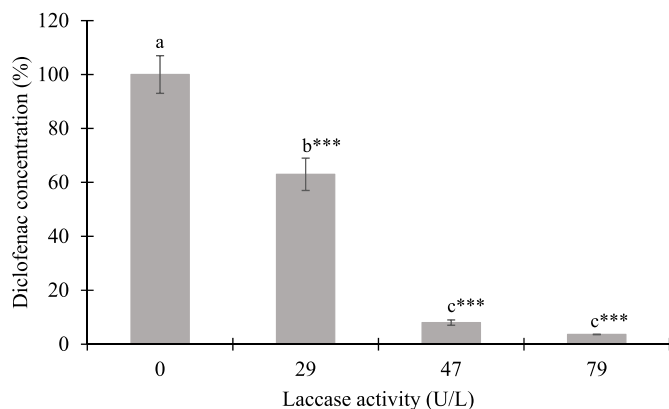


Fig. 3. Residual concentration of diclofenac ($\mu\text{g/L}$) after 5 min of treatment with mushroom substrate colonized by the edible white-rot fungus *Pleurotus ostreatus* and diluted to different levels of laccase activity. Significant difference between the treatments is indicated by different lower-case letters. The level of significance compared to the control is indicated by asterisks, *** $p < 0.001$. Mean \pm standard deviation are shown, $n = 3$.

Table 1

Residual concentration of test pharmaceuticals ($\mu\text{g/L}$) after 5 min of treatment. Laccase activity in the treatment based on mushroom substrate was 58 U/L. No laccase activity was observed in the controls. Mean \pm standard deviation are shown, $n = 3$.

Pharmaceutical	Control without substrate	Control with uncolonized substrate	Mushroom-colonized substrate
Bicalutamide	2.2 \pm 0.2 a ^a	1.4 \pm 0.1 b	0.8 \pm 0.04 c
Lamotrigine	1.5 \pm 0.1 a	1.1 \pm 0.1 b	0.3 \pm 0.01 c
Metformin	62.3 \pm 2.1 a	12.7 \pm 0.6 b	5.2 \pm 1.3 c

^a Values within rows followed by different letters are significantly different ($p < 0.05$).

4. Discussion

4.1. OMPs removal after treatment

Significant removal was observed for all test compounds after treatment in this study, clearly demonstrating the potential of using mushroom substrate colonized by *P. ostreatus* for removal of OMPs from wastewater. Degradation of diclofenac by laccase has typically been studied previously using purified enzymes or submerged cultivation of laccase-producing fungi (Yang et al., 2017). To the best of our knowledge, the effect of direct use of colonized mushroom substrate on diclofenac concentrations in water has not been reported previously. In contrast to previous laccase degradation experiments on diclofenac which are reviewed in Yang et al. (2017), in this study we observed high removal of diclofenac (80–90% reduction in concentration) after a very short period (5 min) of exposure to colonized mushroom substrate. To the best of our knowledge, no previous study has examined laccase-mediated degradation of bicalutamide, lamotrigine, and metformin in wastewater. However, for lamotrigine a recent study reported almost complete removal by *P. ostreatus* after 20 h of fungal growth and cited enzymatic degradation as a potential explanation for this removal (Chefetz et al., 2019). Again, an interesting finding in the present study compared with that study is the short period needed for significant removal when using colonized mushroom substrate (5 min compared with 20 h). As mentioned in the introduction have some compounds, such as small phenolic substances, been shown to increase the reaction rate of laccases (Yang et al., 2017). It is possible that use of lignocellulosic mushroom substrate creates an environment where such compounds are present, as they are abundant in wood (Bulian and Graystone, 2009).

Our results support previous findings that SMS obtained from commercial cultivation of *P. ostreatus* can efficiently degrade OMP (bisphe-nol A) in wastewater (Kresinová et al., 2018) and that extract of SMS from the related species *P. pulmonarius* can achieve removal of chloro-thanil from wastewater (Córdova Juárez et al., 2011). Use of SMS from production of *P. ostreatus* for treatment of water contaminated with various dyes has also been studied (Papinutti and Forchiassin, 2010; landolo et al., 2011). Decolorization and detoxification of the contaminated waters was observed in those studies, an effect partly attributed to adsorption of the dyes on the SMS. In the present study, the larger particles (~ 1 mm) were removed by filtration and no effect of the remaining particles on diclofenac removal was observed. For bicalutamide, lamotrigine, and especially metformin, a significant effect of treatment with uncolonized mushroom substrate was observed. This was most probably due to adsorption of the compounds to the remaining particles, indicating that the particles should be collected after treatment. Thus, several mechanisms are involved in OMPs removal using mushroom substrate including enzymatic degradation performed by fungal ligninolytic enzymes and adsorption to particles.

The main degradation mechanisms of the ligninolytic enzymes include extracellular oxidation by laccases and peroxidases and the intracellular degradation involving for example, cytochrome P450 monooxygenases and nitroreductases, which allow either the catabolisation through oxidation and/or reduction or the conjugate formation by transferases (e.g. O-glucoside or O-glucuronide). These reactions can lead to the complete mineralization of the compounds or just to the excretion of metabolites (Harms et al., 2011). Biodegradation processes are required not only to remove the target compound, but also potentially toxic metabolites remaining after degradation. Laccase-mediated degradation of diclofenac, using laccases from the white-rot fungi, has been studied by both Hahn et al. (2018) and Alharbi et al. (2019). In both these studies, enzymatic transformation products of diclofenac were observed suggesting that further studies on the biodegradability of these transformation products are needed. However, it is promising that both studies reported a clear decrease in toxicity of the laccase-treated effluent.

4.2. Laccase activity in substrate colonized by *P. ostreatus* over time

In the present study, increased removal of diclofenac was observed on increasing the laccase activity from 30 U/L to 50 U/L. Thus, conditions allowing high activity of laccase are desirable. Our data suggest maximal activity of 0.8 U laccase per g of mushroom substrate. A value of 0.5 U laccase per g mushroom substrate has been reported previously (Papinutti and Forchiassin, 2010), in a study using similar enzyme substrate and the same species as in the present study. However, the method e.g., homogenization, fungal strain, mycelial growth phase, and composition of the mushroom substrate will have an impact on laccase activity.

It is generally reported that laccase expression is regulated by nutrient availability, being produced in higher concentrations in response to lack of important nutrients such as nitrogen (Kües and Rühl, 2011). However, considerable differences between species and nutrient sources (i.e., inorganic or organic nitrogen) have been reported for laccase expression (Kües and Rühl, 2011; Economou et al., 2017). In the present study we used substrate based on sawdust and wheat bran, which is commonly used in commercial production of edible white-rot fungi. Furthermore, the cultivation was performed under conditions similar to those in commercial production systems (Stamets, 2000). Our findings of 90% biological efficiency, based on harvest of the first flush, and high protein content of the fruiting body demonstrate that our production system is comparable to standard production. Our results suggest maximum laccase activity of mushroom substrate in the initial phase of fruiting and the lowest activity after mushroom harvest, with an approximately 10-fold variation in laccase activity over the study period. The finding of a gradual decrease in laccase activity until harvest

(during growth of the fruiting bodies) and a slight increase in laccase activity in the post-harvest period (before initiation of a second fructification) is confirmed in previous research (Kurt and Buyukalaca, 2010; Sugimoto et al., 2001). This suggests a role of the physiological state of *P. ostreatus* in exudation of laccases and that the enzyme has an important role in mycelial growth and nutrient acquisition before fructification.

4.3. Considerations on use of substrate colonized by *Pleurotus ostreatus* for OMPs removal

Oyster mushrooms are one of the top three cultivated mushrooms worldwide, and colonized substrate is easily available (Royse et al., 2017). From a practical perspective, direct use of colonized substrate would be less resource-intensive than downstream processing and purification of the enzymes. It would also decrease the need for providing suitable conditions for fungal growth in wastewater, such as addition of carbon and nitrogen sources to stimulate growth and laccase expression. However, use of SMS might not be the best choice for treatment of contaminated wastewater, as the laccase activity is lower in SMS than in the colonized mushroom substrate immediately before initiation of fruiting body formation. This implies that the alluring prospect of combined production of mushroom and laccase-producing substrate when developing this concept for wastewater treatment might not be optimal. However, production of colonized substrate directly at the wastewater treatment plant is a viable option.

The mycelial growth phase of *P. ostreatus* is fast and easy to achieve at room temperature in closed containers. The following step, initiation and growth of fruiting bodies, is more technically challenging, due to the need for accurate regulation of temperature, light, and humidity (Stamets, 2000). However, based on our findings, it could be excluded from the process. Furthermore, substrates produced locally at the wastewater treatment plants, such as wetland biomass, could be used for propagation of the mycelium (Hultberg et al., 2018). Considering the short period needed for OMPs removal, possible adsorption of OMPs to particles, and the need for sufficient levels of dissolved oxygen in the reaction (Munk et al., 2017), a trickling filter with a bed of colonized mushroom substrate could be of use in wastewater treatment plants. There are, however, several aspects of concern in this scenario, such as the impact of the organic carbon present in the wastewater on laccase activity, durability of laccase exudation and life span of the filter, which need to be further examined.

Compared with other commonly cultivated white-rot fungi, such as shiitake mushroom (*Leninula edodes*), *P. ostreatus* shows aggressive growth and high capability to outgrow naturally occurring microorganisms. From a practical point of view, only pasteurization is needed before inoculation with *P. ostreatus*, whereas other fungi demand energy-intensive pretreatment of the substrate, such as sterilization before inoculation followed by successful colonization (Stamets, 2000; Sánchez, 2010). Thus, in production of colonized substrate at the wastewater treatment plant, only a mild pretreatment of the substrate would be needed before fungal inoculation. It can also be assumed that the raw material (substrate) is cheap and potentially locally available. Furthermore, transport and storage of SMS, which has been demonstrated to impair the efficiency in reducing pollutants (Córdova Juárez et al., 2011), would be avoided. Spore production, an health issue in the mushroom industry due to allergic reactions (Vereda et al., 2007), would also be avoided when no fruiting bodies are produced.

5. Conclusion

We conclude that significant removal was observed for all test compounds after a short period of treatment with mushroom substrate colonized by *P. ostreatus*. Thus, this study demonstrates the potential of using mushroom substrate for removal of OMPs from wastewater. The direct use of colonized substrate for bioremediation of wastewater

would be less resource-intensive than downstream processing and purification of laccases and SMS is easily available as a waste product after commercial mushroom production. However, SMS might not be the best choice for treatment of contaminated wastewater, as the laccase activity is considerably lower compared to colonized mushroom substrate before development of fruiting bodies. Considering the results obtained in the present study, colonized mushroom substrate could be of possible use in wastewater treatment plants for bioremediation purpose e.g. as a filter material. There are, however, several aspects that need to be further studied, such as duration and amount of laccase exudation over time by the colonized substrate.

Declaration of competing interest

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

M. Hultberg: Conceptualization, Methodology, Formal analysis, Investigation, Funding acquisition, Writing - original draft. **L. Ahrens:** Resources, Supervision, Writing - review & editing. **O. Golovko:** Investigation, Methodology, Validation, Writing - original draft.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2020.111087>.

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Ethical approval

This work did not involve any studies with human participants or animals performed by any of the authors.

Informed consent

All authors have the authority to publish this material and have agreed to submit it to the Journal of Environmental Management. Compliance with ethical standards.

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