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Spawn-based pellets of *Pleurotus ostreatus* as an applied approach for the production of laccase in different types of water

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ABSTRACT

In recent years, oxidoreductase enzymes such as laccases have received considerable attention for their ability to degrade and eliminate organic micropollutants from contaminated water in a process known as enzyme-based wastewater treatment. Thus, methods to produce high laccase activity in water are a point of focus, with white-rot fungi being highlighted as a tool in this context. This study, therefore, explored the applied approach of direct addition of mushroom spawn of the white-rot fungi *Pleurotus ostreatus* into water and its potential for laccase production under different conditions. Grain spawn was observed to be preferable to sawdust spawn, resulting in laccase activity of 53.9 ± 5.9 U/L and 4.8 ± 0.8 U/L, respectively. Laccase activity was induced by adding kraft lignin (4 g/L), and an eightfold increase to 446.3 ± 43.1 U/L was observed for grain spawn. Lignin accumulated in the spawn over time, resulting in brown pellets composed of spawn, mycelium and lignin. Our results demonstrated that high levels of laccase activity could be obtained in different types of water, including effluent municipal wastewater, using this method. No impact from the addition of inorganic nitrogen (ammonium nitrate, N-levels 14 mg/L, 140 mg/L) or organic nitrogen sources (urea, yeast extract, wheat bran, N-levels 14 mg/L, 140 mg/L) was observed for the treatment with grain spawn and lignin, suggesting that stable laccase activity can be expected under these nutritional conditions.

1. Introduction

White-rot fungi are crucial in natural bio-oxidation processes in terrestrial environments, primarily targeting lignin in plant structures (Mohammadi et al., 2022). The fungi release extracellular enzymes to degrade complex polymers and absorb the smaller molecules as nutrients. Important enzymes include laccase and lignin-modifying peroxidases, which act synergistically during lignin degradation (Mir-Tutusaus et al., 2018). The enzyme laccase (EC 1.10.3.2) can be obtained from other sources besides fungi, such as plants, bacteria and insects (Arregui et al., 2019; Gasser et al., 2014). However, fungal laccases have received much attention due to their low substrate specificity and capacity to transform diverse compounds such as Polycyclic Aromatic Hydrocarbons (PAHs) and Polychlorinated Biphenyls (PCBs) (Dinakarkumar et al., 2024). The oxidation reactions facilitated by laccases produce free radicals which assist in producing mediators which can then react with a wide range of high-redox potential substrates (Zhuo and Fan, 2021).

The secretion patterns of enzymes vary among different white-rot species; the common oyster mushroom (*Pleurotus ostreatus*) has a dominance of laccases when exposed to lignocellulosic substrate (Fernández-Fueyo et al., 2016). This is also one of the most widely cultivated and appreciated mushrooms worldwide. Thus, this species is well-known and safe to use in large-scale processes. The production of laccases by *P. ostreatus* is affected by culture conditions, including the type of carbon and nitrogen source, the ratio of carbon to nitrogen, and the pH of the fermentation medium (Chiranjeevi et al., 2014). As well as the addition of various supplements, specific compounds such as lignin and some metal ions can significantly increase the production and activity of laccase (Yang et al., 2017).

Laccases have a wide substrate range, and only use oxygen as the final electron receptor; thus, these enzymes are very attractive for a broad range of industrial and environmental purposes (Antecka et al., 2021; Lettera et al., 2011). In recent years, laccases have received considerable attention for their ability to degrade and eliminate organic micropollutants from contaminated water in a process known as enzyme-based wastewater treatment (Mohammadi et al., 2022; Unuofin et al., 2019; Arregui et al., 2019; Sá et al., 2022). In this context, several different experimental approaches have been carried out, including the

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use of purified enzymes. Laccase production is considered part of secondary metabolism, resulting in a long production cycle, which obstructs industrial laccase production (Yang et al., 2017). Also, spent mushroom substrates have been tested as a low-cost approach, but this has led to a fast wash-out of the enzymes (Hultberg and Golovko, 2024). Considering the potential of laccase for the removal of micropollutants, it is of interest to look into new approaches for the production of this enzyme in water. The present study, therefore, explored the innovative and applied approach of direct addition of mushroom spawn of *P. ostreatus* into water and the potential it posed for laccase production in situ. Different types of water and nutritional conditions, with nitrogen in focus, were studied. This is of interest as repression of laccase production has been reported in respect to increasing levels of nitrogen (Durán-Sequeda et al., 2021). As laccase is of interest for enzyme-based wastewater treatment, nitrogen was supplied in concentrations relevant for effluent municipal wastewater.

2. Material and methods

2.1. Microorganism and cultivation

The experiments were performed with the white-rot fungal species *Pleurotus ostreatus* M2191 which was obtained from the company Mycelia BVBA, Deinze, Belgium. This species is used in commercial production of fruiting bodies of oyster mushrooms. Two different types of spawn, grain spawn and sawdust spawn, were used in the experiments. Grain spawn was obtained from Mycelia BVBA, Belgium, and sawdust spawn was produced through the inoculation of 3 % (weight (w)/w) of the grain spawn in sterilized sawdust (Birch, 2–4 mm) with a moisture content of 65 %. The inoculated sawdust was cultivated for 12 days at 22 °C before use in experiments. These spawn types provide the fungus with different nutritional conditions and may impact the fungal growth. The total amount of carbon and nitrogen in grain spawn and sawdust spawn (dry weight, dw) were determined before the start of the experiments using a Vario Max CN Element Analyzer. The grain spawn had a C/N ratio of 10:1 while the spawn based on sawdust (Birch, 2–4 mm) had a C/N ratio of 214:1. Thus, propagation on grain spawn can be expected to give the fungus considerably higher access to nitrogen compared to when propagated on sawdust. In the experiments, the spawn was added in a concentration of 40 g/L (wet w) and cultivated in Erlenmeyer glass flasks in a horizontal orbital shaker (VWR, Advanced 5000 Shaker, Radnor, PA, USA) operated at 100 rpm at room temperature (20–22 °C). The cultivation of the spawn under submerged conditions was performed for 3 days (72 h).

2.2. Experimental set-up

Experiments were performed in sterile distilled water, synthetic wastewater (OECD 303 A, 2001) and in effluent municipal wastewater provided by a local wastewater treatment plant as described in detail in Table 1. The effluent municipal wastewater had a pH of 7.5, a total nitrogen content of 25.5 ± 0.7 mg/L, total phosphorus below 0.05 mg/L and COD (chemical oxygen demand) of 31.3 ± 1.5 mg/L. A volume of 25 ml of liquid was used in each replicate and the experiments were set up with three replicates.

In the first experiment, kraft lignin (Sigma-Aldrich 370,959) was added to the flasks with synthetic wastewater in concentrations 0, 1, 2, 4 and 16 g/L to study the potential for inducing the production of ligninolytic enzymes. The experiments were performed at near-neutral pH, ranging between 6.5 and 7.5. At this pH, kraft lignin is considered insoluble (Sewring et al., 2019), and any potential, very small solubility was not determined in this study. This experiment was performed with grain spawn (40 g/L) and both laccase and manganese peroxidase (MnP) activity were monitored daily for 3 days. A supplementary experiment using lignin in the concentrations of 0 and 4 g/L and grain spawn (40 g/L) was performed over a longer time period (11 days). Samples were taken over time to determine enzyme activity and impact on pH. In order to determine if it was possible for fungus to grow and produce enzymes in real wastewater, an additional experiment was performed in effluent municipal wastewater provided by a local wastewater treatment plant. In this experiment, lignin was added in a concentration of 4 g/L and laccase and MnP activity as well as impact on pH was monitored daily for 3 days.

The impact of nutritional amendments and spawn type on laccase activity was studied in a similar set-up as described above. These experiments were performed using sterile distilled water, with and without the addition of lignin (4 g/L). Only laccase activity was monitored as MnP activity was absent or recorded in levels below 1 U/L in the previous experiments. Grain spawn and sawdust spawn were evaluated and used at a concentration of 40 g/L. The tested amendments were ammonium nitrate (NH_4NO_3), urea ($CO(NH_2)_2$), yeast extract (Duchefa, Haarlem, The Netherlands) and wheat bran. The amendments were added in two different concentrations (Table 1) which were based on the nitrogen content of the compounds. The tested concentrations of nitrogen, 14 and 140 mg/L, were selected based on expected nitrogen levels in effluent wastewater. The yeast extract contained 10 % nitrogen based on its dry weight according to the manufacturer. The nitrogen content in the solid nitrogen source, wheat bran, was determined as 1.8 ± 0.1 % using a Vario Max CN Element Analyzer.

2.3. Enzyme analysis

Laccase activity and MnP activity was analysed colorimetrically by detecting the oxidation product 2,6-dimethoxyphenol (DMP), following the method described by Parenti et al. (2013), which utilized a molar extinction coefficient (ε468) of 49,600 M^{-1} cm^{-1} for DMP. The replicates were sampled directly and the reaction mixture was comprised of 0.45 ml of dilute sample and 0.5 ml of 10 mM 2,6-dimethoxyphenol (DMP) in a 100 mM acetate buffer (pH 5). Absorbance readings were taken at 468 nm. Enzyme activity was quantified, with one unit (U) defined as the production of 1 μmol of product per minute. MnP activity was initiated through the addition of $MnSO_4$ and fresh H_2O_2, as

Table 1
The used conditions and measurements in the experiments performed with spawn pellets of *Pleurotus ostreatus*. Abbreviations: Nitrogen (N); laccase (lac); Manganese peroxidase (MnP).

Type of water	Type of spawn	Lignin	Amendment	Concentration	Measurements
Synthetic wastewater	Grain	0–16 g/L	none	–	Lac/MnP
Municipal wastewater	Grain	4 g/L	none	–	Lac/MnP
Sterile distilled water	Grain/Sawdust	0 g/L, 4 g/L	Ammonium nitrate	0.5 mM (14 mg/L N)	Lac
		0 g/L, 4 g/L		5 mM (140 mg/L N)	Lac
Sterile distilled water	Grain/Sawdust	0 g/L, 4 g/L	Urea	0.5 mM (14 mg/L N)	Lac
		0 g/L, 4 g/L		5 mM (140 mg/L N)	Lac
Sterile distilled water	Grain/Sawdust	0 g/L, 4 g/L	Yeast extract	140 mg/L (14 mg/L N)	Lac
		0 g/L, 4 g/L		1400 mg/L (140 mg/L N)	Lac
Sterile distilled water	Grain/Sawdust	0 g/L, 4 g/L	Wheat bran	800 mg/L (14 mg/L N)	Lac
		0 g/L, 4 g/L		8 g/L (140 mg/L N)	Lac

described by Field et al. (1996), with peroxidase activity corrected for background laccase activity.

2.4. Statistical analysis

All experiments were conducted with three replicates, and the mean values along with their corresponding standard deviations are reported. The data were analysed through the analysis of variance (ANOVA), followed by Tukey's test or by *t*-test. Statistical significance was determined at a significance level of $p < 0.05$. The statistical analysis was conducted using Minitab version 19 (Minitab® 19.2020.1).

3. Results

3.1. Spawn-based pellets and enzyme production

Pellets of an approximate size of 0.3–0.8 cm, composed of the grain spawn and mycelium of *P. ostreatus* growing out from the spawn, developed in the synthetic wastewater (Fig. 1). The addition of lignin was found to induce laccase activity (Fig. 2). On day 3, significantly higher laccase activity was recorded for the treatments receiving ≥2 g lignin per L than in wastewater without the addition of lignin. MnP activity was not detected in any of the treatments in this experiment. The addition of lignin to the wastewater initially resulted in an opaque and dark brown suspension, but over time, lignin accumulated in the mycelium, resulting in brown pellets composed of mycelium and lignin and transparent water with slight brownification on day 3 (Fig. 1, Supplementary Fig. 1). However, in the treatments with the highest concentration of lignin (16 g/L), the wastewater remained opaque and dark brown over time. The laccase activity in the treatments receiving 4 and 16 g lignin per L did not differ significantly. Thus, 4 g/L of lignin was used in the following experiments.

When comparing enzyme activity and pH over time in the treatment receiving 4 g/L of lignin and in the control without the addition of lignin (Fig. 2), there was a low level of MnP activity (<1 U/L) in the treatment without added lignin in the first 24 h. After this period, MnP activity was not seen in any of the treatments. Laccase activity was already significantly higher compared with the control after just 24 h. The addition of lignin had little impact on the pH of the wastewater, with an initial pH of 7.2 ± 0.04 for the synthetic wastewater and 7.3 ± 0.03 for the synthetic wastewater with added lignin (4 g/L). During fungal growth, a slight decrease in pH was noted for both treatments during the first three days, with the lowest pH recorded being 5.2 (Fig. 2). The treatment with grain

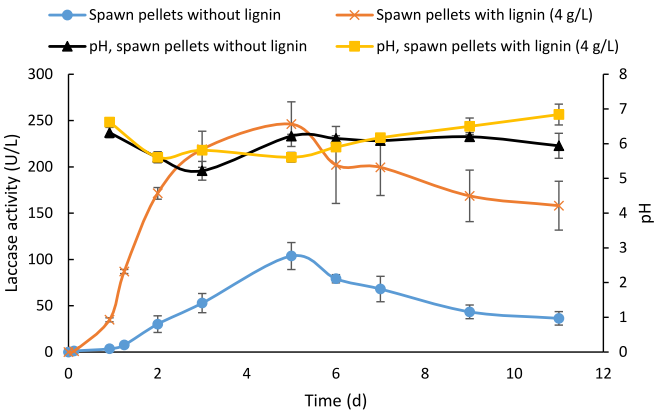

Fig. 2. Laccase activity and pH over time in synthetic wastewater, with and without lignin (4 g/L), in treatment with pellets of *Pleurotus ostreatus*. Mean ± std., $n = 3$.

spawn (40 g/L) and lignin (4 g/L) were also tested in effluent municipal wastewater, and laccase activity and impact on pH was followed over time. The obtained results were similar to those in synthetic wastewater considering the size of pellets, accumulation of lignin, impact on pH and laccase activity. The background laccase activity in the effluent municipal wastewater was below 1 U/L. After 24 h of fungal growth, laccase activity was 228.6 ± 59.6 U/L, and after three days of fungal growth, it was 499.6 ± 20.6 U/L. MnP activity was not detected in this water.

3.2. Impact of nutritional factors on laccase production of spawn

In order to gain additional information about the impact of nutritional conditions on laccase production of the spawn pellets, experiments were performed in distilled water, with spawn from sawdust and grain included. The use of grain spawn resulted in considerably higher laccase activity in water compared to sawdust spawn. For the treatments without lignin, an approximately ten times higher activity was recorded when grain spawn was used, 4.8 ± 0.8 and 53.9 ± 5.9 U/L, respectively. The impact of the addition of lignin was also considerably stronger for the grain spawn, with an eightfold increase from 53.9 ± 5.9 to 446.3 ± 43.1 U/L observed. For the sawdust spawn, the addition of lignin resulted in slightly under a doubling, from 4.8 ± 0.8 to 8.3 ± 1.5 U/L. It should also be noted that for the sawdust spawn, the water remained

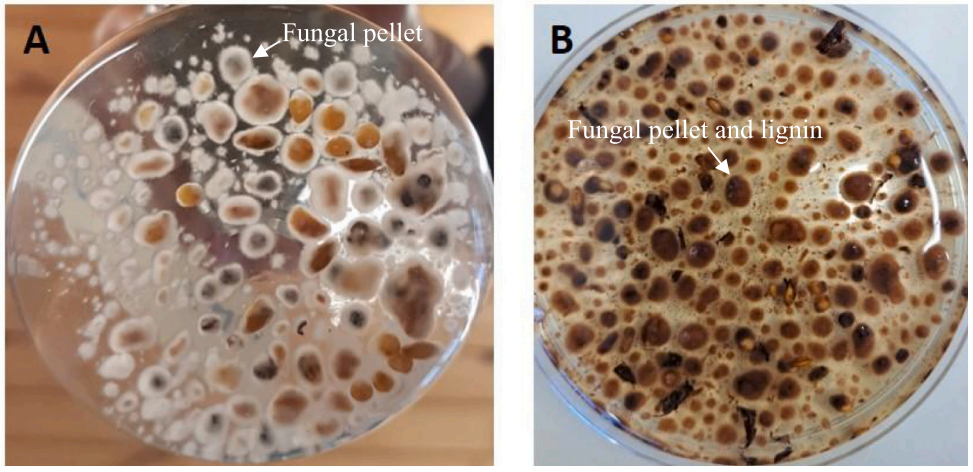

Fig. 1. (A) Fungal pellets (*Pleurotus ostreatus*) formed in wastewater through hyphae which grew out from the grain spawn. The addition of lignin (4 g/L) to the wastewater initially resulted in an opaque, dark brown suspension, but, over time, lignin accumulated in the mycelium, resulting in (B) brown pellets composed of both mycelium and lignin. The spawn had been growing for three days in both images. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

opaque and dark brown after the addition of the lignin; thus, no accumulation of lignin in the mycelium was observed.

When the impact of adding different nitrogen sources was studied in grain spawn, it was clear that none of the added compounds had any effect on the grain spawn when lignin was added (Fig. 3). For the grain spawn which was not exposed to lignin, a significant increase was observed after the addition of urea, yeast extract and wheat bran in the highest concentration (N concentration of 140 mg/L). For the sawdust spawn, a slightly more variable pattern was observed (Fig. 4). A significant decrease in laccase activity was observed in the treatment with sawdust spawn which was not exposed to lignin when ammonium nitrate was added. A significant increase in laccase activity was observed for all treatments exposed to wheat bran. This increase was remarkably high in the treatment with sawdust spawn and lignin. The control treatment with lignin had laccase activity of 8.3 ± 1.5 U/L, while the addition of wheat bran in the highest concentration resulted in laccase activity of 134.3 ± 21.5 U/L. Also, the addition of urea and yeast extract in the highest concentration resulted in significantly higher laccase activity in the treatments with sawdust spawn and the addition of lignin.

4. Discussion

The fungal strain that is used in the present study, *P. ostreatus* M2191, has previously been applied in bioremediation experiments studying the removal of pharmaceuticals in water. Focusing on the substance diclofenac, a removal of more than 90 % was observed after 5 min of exposure to an enzyme suspension with laccase activity of approximately 100 U/L. That study also confirmed that decreasing laccase activity in the enzyme suspension resulted in decreased removal of diclofenac (Hultberg et al., 2020). A later study, performed on a mixture of pharmaceuticals, confirmed that laccase activity in the enzyme suspension is of importance for pharmaceutical removal (Hultberg and Golovko, 2024). Thus, high levels of laccase activity in water are searched for to optimize removal of organic micropollutants from water.

In both studies mentioned above, the enzyme suspensions were prepared from a sawdust substrate colonized by *P. ostreatus* M2191. This approach is labor-intensive and time-consuming, and the present study therefore explored a different method, the development of laccase-producing fungal pellets directly in water. Our results demonstrate that high levels of laccase activity can be obtained in different types of water, including effluent municipal wastewater, using this method. This can be explained by the fact that mushroom spawn represents an actively growing fungal culture on a substrate (grain or sawdust) which is well-suited for the fungus. Thus, the fungus is not initially dependent on recovering nutrients from its surroundings. The fungal hyphae can grow out of the spawn and release enzymes, such as laccase, which are of importance to recover new nutrients (Balan et al., 2022). On the other hand, an important limiting factor, considering fungal growth and enzymes release in the system described in this study, is the oxygen concentration in the water.

When growing white-rot fungi in wastewater, different amendments can be added to the water in order to induce enzyme activity, as described by Parenti et al. (2013). Due to the many potential industrial applications of laccases, their production has been studied in detail and several important inducers of laccase production have been identified. A well-explored option for increasing laccase activity is through the addition of inducers such as metals, especially copper ions, and aromatic compounds (Zhuo and Fan, 2021). However, in the present study, a future scenario of increased laccase activity in the treatment of wastewater was considered, which would require the use of an environmentally friendly and low-cost compound as an inducer. Lignin is currently of great interest for the development of different types of 'green' materials (Gendron et al., 2022; Österberg et al., 2020) and has also been shown to strongly induce laccase production in *P. ostreatus* (An et al., 2018). Kraft lignin (alkali lignin) is available in large quantities as a solid waste product from the pulp and paper industry (Argyropoulos et al., 2023), and was therefore considered as a suitable inducer of laccase production in the present study. In preliminary studies, it has been confirmed that addition of kraft lignin (1 % dwt/dwt) to spent mushroom substrate of *P. ostreatus* significantly increased the amount of laccase that could be extracted from the substrate (data not shown). Thus, considering a situation where kraft lignin and SMS is available (e.g. as waste sources), this could be further investigated as sustainable and low-cost method for extraction of laccase.

The observed accumulation of lignin in the growing mycelium, as shown in Fig. 1, is an interesting finding. It is beneficial from a water treatment perspective, as the small lignin particles will largely be removed from the wastewater. The fungi-lignin pellets developed in this study were comparable to the algal-fungal pellets produced in studies of fungal-assisted algal harvesting (Zhang and Hu, 2012; Lin et al., 2022). The color differed evidently (brown versus green), but the size and efficiency in removing particles/cells from the suspension showed similarities. In fungal-assisted algal harvest, the surface charge, with negatively charged algal cells and positively charged fungal hyphae, is an important mechanism behind pellet formation (Lin et al., 2022). Surface charge may also partly explain the accumulation of lignin in fungal mycelium in our study, as kraft lignin has a negative surface charge (Norgren and Lindström, 2000). The strain of *P. ostreatus* used in

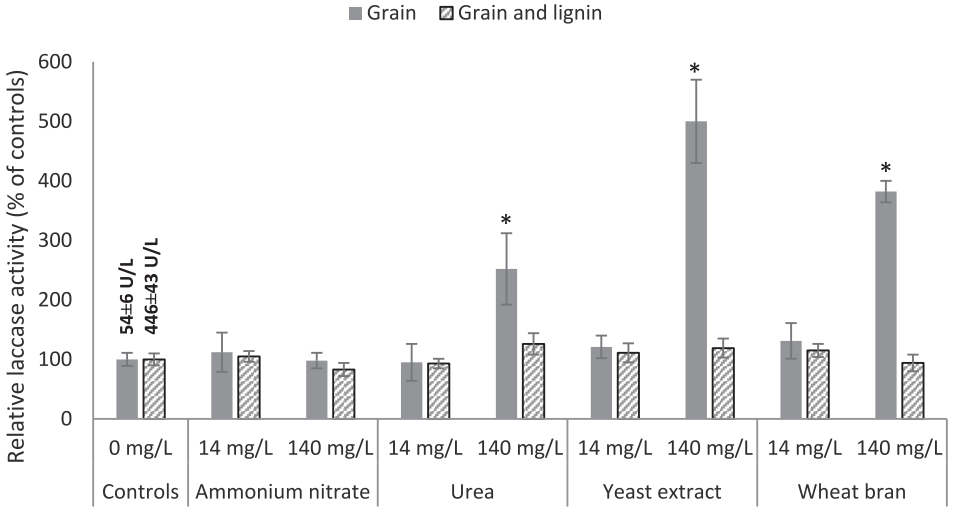

Fig. 3. The impact of nutritional amendments on laccase activity in water by grain spawn pellets of *Pleurotus ostreatus* relative to control (%). Laccase activity (U/L) in the controls are presented in the figure. Significant differences compared to the controls are indicated with an asterisk (*). Mean ± std., $n = 3$.

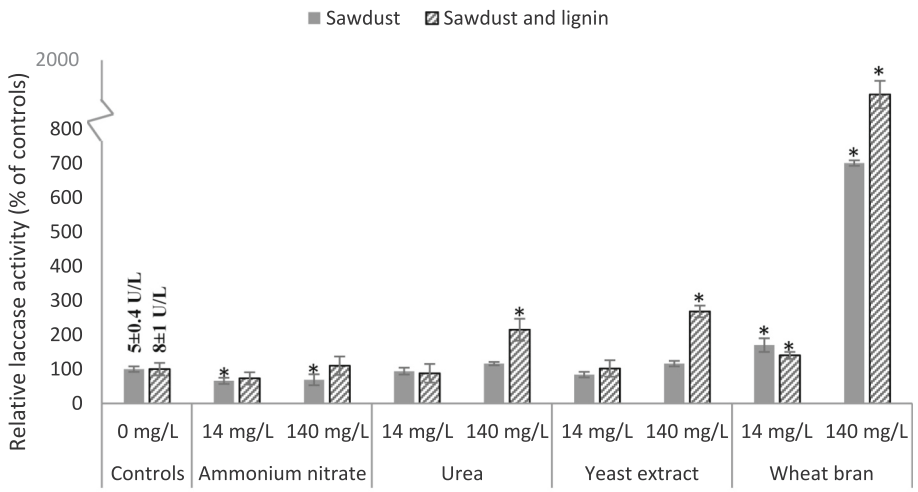

Fig. 4. The impact of nutritional amendments on laccase activity in water by sawdust spawn pellets of *Pleurotus ostreatus* relative to control (%). Laccase activity (U/L) in the controls are presented in the figure. Significant differences compared to the controls are indicated with an asterisk (*). Mean ± std., n = 3.

this study has previously been tested for fungal-assisted algal production, but no accumulation of the microalga (*Chlorella vulgaris*) in the mycelium was observed (Hultberg et al., 2020).

The concept developed in this study can be described as spawn-based fungal pellets with induced production of laccase. Spawn is an essential component in commercial mushroom production, is completely non-toxic and has a well-established production chain (Balan et al., 2022). Thus, from an environmental perspective, its large-scale use for water treatment is an option. A few different spawn types are available for mushroom growers and, by extension, for water treatment. The common types are sawdust spawn, grain spawn, liquid spawn, and stick spawn (Zhang et al., 2019). Sawdust spawn is frequently used and has a low cost. The use of grain spawn is also widespread, providing excellent nutritional conditions for propagation of the fungi but is associated with slightly higher costs and contamination rates compared to sawdust (Rosado et al., 2002). From the results comparing sawdust- and grain spawn, it was clear that grain spawn of *P. ostreatus* exhibited better laccase activity when grown in water (Fig. 3 and Fig. 4). This finding can most likely be explained by the fact that grains provide a more favourable substrate compared to sawdust. This is partly reflected in the difference in C/N ratio between the spawn types and the fungus having access to considerably more nitrogen when produced in grain compared to sawdust.

It should be pointed out that nitrogen, a vital element for all living organisms, is never added alone when supplied as a nutrient source. This fact is particularly applicable to organic nitrogen sources, which also act as a carbon source from a fungal perspective. The addition of an additional carbon source may enhance fungal growth, resulting in increased biomass and thereby leading to increased production of enzymes. In the present study, the aim was to optimize laccase activity in water, irrespective of fungal biomass production. Thus, mycelial growth was not measured, and the measured laccase activity can be related both to a specific effect on laccase production and to biomass production. Despite this limitation, some conclusions which are of importance for the further development of spawn-based pellets in water can be drawn. First, for grain spawn with access to lignin, none of the added amendments had any impact on laccase activity. Thus, the conditions created through the addition of lignin and the nutrients provided by the grain spawn were favourable for high laccase activity, irrespective of the nutrients in the surroundings. This is of interest as repression of laccase production has been reported in respect to increasing levels of nitrogen (Durán-Sequeda et al., 2021). From the conditions provided in this study, it seems reasonable to conclude that considering effluent wastewater with total nitrogen levels not exceeding 140 mg/L, stable laccase activity could be expected. The repression of laccase activity through the addition of nitrogen was observed only for the sawdust spawn in combination with ammonium nitrate.

Overall, the addition of ammonium nitrate as a nitrogen source performed poorly in comparison to the organic nitrogen sources. In previous studies on this topic, the impact of nitrogen sources on laccase activity in submerged cultures of *P. ostreatus* has had both similar and differing results reported (Mikiashvili et al., 2006; Stajic et al., 2006). As discussed by Durán-Sequeda et al. (2021), laccase production in fungi is subjected to several regulatory levels, and this is part of an explanation for the wide range of laccase activity reported in the literature. From our study, a remarkably high increase of laccase activity was observed for the sawdust spawn when exposed to lignin and wheat bran. The nutritional conditions supplied in this treatment resemble common conditions applied in solid-state fermentation for the fruiting body production of *P. ostreatus*. Still, the measured activity was considerably lower, 134 U/L versus 446 U/L, compared to the use of grain spawn exposed to lignin. It is, however, of interest to note the use of a solid substances such as wheat bran had a clear impact on laccase activity in submerged liquid cultivation too.

In this study, the focus was on developing an applied method for the production of laccase in water, especially considering enzyme-based wastewater treatment. Our results clearly show that high laccase activity can be obtained in effluent municipal wastewater. However, in this type of wastewater, the use of lignin for the induction of laccase activity may result in increased levels of organic carbon in the water, which will require additional treatment before release. In future research, it is therefore of interest to explore other measures for induction of laccase while still considering that the environmental impact of any amendment must be low. Potentially, the strategy based on autoinduction suggested by Lettera et al. (2011) is of interest to explore. In addition, laccase production of the spawn-based pellets could be explored in wastewater from earlier steps in the treatment process where the increase in organic carbon would be less of a problem. This approach would, however, expose the fungus to a more complex and challenging environment compared to growth in effluent wastewater.

5. Conclusion

The present study explores an applied approach for the production of laccase in water. Commercial mushroom spawn of the edible mushroom *Pleurotus ostreatus* was used to develop white-rot fungal pellets in water. Lignin was used to induce laccase production and was observed to accumulate in the pellets, composed of grain spawn, mycelium and

lignin, which developed in all tested waters. In previous experiments, high removal of the pharmaceutical diclofenac has been demonstrated using an enzyme suspension, produced by the same strain as used in this study, with laccase activity of approximately 100 U/L (Hultberg et al., 2020). Based on the method described in this study, laccase activity exceeding 400 U/L could be obtained in effluent municipal wastewater. Thus, the reported findings may have implications for enzyme-based wastewater treatment. In addition, this method may also provide a straight forward approach for production of laccase based on grain spawn, lignin and water. In order to determine if the described method is a viable approach for production of laccase for purification, additional work is needed to determine the cost and amount of labor needed to extract the enzyme from the suspension.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Declaration of generative AI and AI-assisted technologies in the writing process

Generative AI and AI-assisted technologies were not used in the writing process.

CRediT authorship contribution statement

Inoka Sanjeewani Ranamukha Hewage: Writing – original draft, Investigation, Formal analysis. **Oksana Golovko:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Malin Hultberg:** Writing – original draft, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mimet.2025.107092.

Data availability

Data will be made available on request.

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