Folia Hort. 35(1) (2023): 25–31

DOI: 10.2478/fhort-2023-0002

FOLIA HORTICULTURAE

Published by the Polish Society for Horticultural Science since 1989

ORIGINAL ARTICLE | Open access | https://sciendo.com/journal/FHORT

Effect of different water contents in the substrate on cultivation of *Pleurotus ostreatus* Jacq. P. Kumm

Lucie Wiesnerová[1,*], *Tereza Hřebečková*[2], *Ivan Jablonský*[1], *Martin Koudela*[1]

[1] Department of Horticulture, Faculty of Agrobiology, Food and Natural Resources, The Czech University of Life Sciences Prague, Prague 165 21, Czech Republic
[2] Department of Agroenvironmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, The Czech University of Life Sciences Prague, Prague 165 21, Czech Republic

ABSTRACT

Pleurotus ostreatus is a widely cultivated and investigated mushroom for its economical and ecological values and medicinal properties. *P. ostreatus* can be cultivated on different lignocellulosic substrates (oak sawdust, wheat straw, corn cobs and many more). Optimal growth is influenced not only by the composition of the substrate but also by the amount of water in it. In our study, *P. ostreatus* was cultivated on wheat straw pellets with different water contents (60%, 65%, 70% and 75%). Mycelium growth, biological efficiency (BE), moisture of substrate, pH, enzymatic activities and relationships were the parameters that were evaluated. Based on the results, the optimum initial substrate water content for mycelial growth and BE of the substrate ranged between 65% and 75%. On the other hand, the highest enzymatic activities of hydrolytic and ligninolytic enzymes (Mn-dependent peroxidase, 1,4-β-glucosidase and cellobiohydrolase) were determined for substrates with 75% of water content.

Keywords: enzymes, mycelial growth, oyster mushroom, pH, wheat substrate

INTRODUCTION

White-rot fungi comprise a large group of organisms known for their ability to produce different types of enzymes for the degradation of lignin and cellulose. Lignin is the main component of wood and other plant biomass, and, together with cellulose and hemicellulose, it is the main source of carbon for the mycelium (Baldrian et al., 2005). One representative of the white-rot fungi is *Pleurotus ostreatus*, which produces ligninolytic and hydrolytic enzymes, such as laccase (EC 1.10.3.2), Mn-dependent peroxidase (EC 1.11.1.13), 1,4-β-glucosidase (EC 3.2.1.21), cellobiohydrolase (EC 3.2.1.91) and many more for the degradation of lignocellulosic substrates (Elisashvili et al., 2001; Mikiashvili et al., 2004, 2006).

Several studies have investigated the relationships among specific enzyme activity and lignocellulose degradation, biological efficiency (BE) and other processes. During cultivation of *Pleurotus* spp., the activities of different enzymes change. For example, the activity of 1,4-β-glucosidase and cellobiohydrolase increases during cultivation, which may be due to the decomposition of cellulose in the later stages of fungal growth (Wang et al., 2001; Baldrian et al., 2005).

*Corresponding author.
e-mail: wiesnerova@af.czu.cz (Lucie Wiesnerová).

The activity of 1,4-β-glucosidase is also affected by other parameters such as the pH of the medium, the temperature and the humidity of the substrate (Goyal and Soni, 2011). Another enzyme involved in the degradation of cellulose is cellobiohydrolase. When cellulose is degraded, the enzymes behave synergistically and produce carbohydrates, which can be easily assimilated by fungal mycelia. The highest activity of cellobiohydrolase was recorded in the phase of primordial deployment and fructification (Velázquez-Cedeño et al., 2002). Manganese-dependent peroxidase (Mn-P) and laccase play an important role in lignin decomposition (Maciel et al., 2012). The first is an extracellular haeme protein containing a carbonyl group and requiring H_2O_2 catalysis with the involvement of Mn^{+}; Mn-P is secreted during mycelium growth and catalyses oxidation, resulting in the formation of phenoxy radicals. Subsequently, a series of non-enzymatic catalysed reactions and further degradation follow (Jin et al., 2021). Laccase is a copper-containing oxidase and can deprotonate hydroxyl groups to form phenoxy anions, leading to the breakage of the side chains of lignin-type structural units (Ihssen et al., 2017). The activity of lignin-degrading enzymes decreases during cultivation (Kannan et al., 1990), and the production of these enzymes depends on the culture medium, pH, temperature and the growth phase of the fungus (Isikhuemhen and Mikiashvilli, 2009; Knop et al., 2015).

An important criterion for the cultivation of *P. ostreatus* is the water content of the substrate as it impacts mycelium growth, yield and enzymatic activities. The optimum water content for *P. ostreatus* cultivation is between 65% and 75%. A high initial water content can cause misshaped fruiting bodies (Wang et al., 2001; Siwulski et al., 2007; Dos Santos Bazanella et al., 2013). Although a high initial moisture level is the best condition for laccase activity, for Mn-peroxidase activity, a low initial moisture level is more suitable (Dos Santos Bazanella et al., 2013).

However, the relationships among enzyme activity, substrate moisture and different substrate layers are still largely unclear. This study is the novel comparison of the activities of multiple enzymes (Mn-dependent peroxidase, 1,4-β-glucosidase and cellobiohydrolase) produced by *P. ostreatus* and their relationships with other parameters, such as pH, water content within different substrate layers at different initial water content of substrate and its impact on mycelial growth and BE of the substrate.

MATERIALS AND METHODS

Mushroom material and spawn preparation

The strain 5081 of *P. ostreatus* (Culture Collection of Basidiomycetes of the Crop Research Institute in Prague, Czech Republic) was used for this experiment. The cultivation medium was prepared according to Pánek et al. (2019), using 2% agar (Carl Roth GmbH, Germany) and 2% malt extract (Carl Roth GmbH, Germany). Cultivation was performed for 7 days at 24°C, and this culture was used for grain spawn preparation. Spawn was prepared according to Košnář et al. (2019). Briefly, wheat grain was cooked for 20 min, dried of surplus water and filled in 1 L bottles. Drained wheat grain was sterilised in an autoclave at 121 °C for 2 hr and subsequently inoculated with pieces of mycelium culture. The inoculated grain was cultivated at 24 °C for 14 days.

Substrate preparation and inoculation

Wheat straw pellets were used for substrate preparation. Pellets were mixed with water to reach moisture levels of 60%, 65%, 70% and 75%, and the substrate was filled in plastic containers (750 g of substrate per container). The prepared substrate was heat treated at 90 °C for 24 hr, cooled down and inoculated with grain spawn (10 g per container).

Cultivation conditions and harvest

The inoculated substrates were kept in an incubation room at 24 °C. Substrate was colonised by the mycelium (14 days from inoculation) and subsequently transferred to a growing room at a temperature of 12°C and a relative humidity of 90%. The fruiting bodies were harvested after 12 days.

pH and water content analysis

Samples were taken from the centre and the upper layer (1–2 cm from the substrate surface) of the substrate. The pH/H_2O was determined using the pH metre Jenway 3505 (Cole-Palmer, United Kingdom), according to BSI EN 15933. The water content was determined using the moisture analyser KERN DAB 200-2 (Kern & Sohn, Balingen, Germany) at 105 °C.

Mycelial growth and BE

Mycelial growth was recorded on four vertical axes around the circumference of the container after 7 days. BE was calculated as the ratio of the weight of the fresh fruiting body (g) per dry weight of substrate (g), expressed as a percentage, according to Liang et al. (2019).

Enzyme analysis

To determine enzyme activities, 0.2 g of the sample was placed in a 50-mL Erlenmeyer flask with 20 mL of acetate buffer, at pH 5.0. Samples were homogenised using an Ultra-Turrax (IKA Labortechnik, Staufen, Germany) at 8,000 rev · min^{-1} for 30 s. To determine the laccase and Mn-peroxidase activities, filtration and desalination were performed. Enzyme activities were measured in 96-well microplates, with 4 wells per sample.

Activities of ligninolytic enzymes (laccase, Mn-dependent peroxidase) were measured spectroscopically at 590 nm as there are changes in absorbance for 3.5 min (measurement 7 × 30 s) in a Tecan Infinite® M200 (Tecan Austria GmbH, Austria). To determine the activity of Mn-peroxidase, a solution of DMAB (25 mmol · L^{-1} 3.3-dimethylamino-benzoic acid), MBTH

(1 mmol · L^{-1} 3-methyl-2-benzothiazolinonehydrazone), $MnSO_4$ (2 mmol · L^{-1}), EDTA Na sol. (2 mmol · L^{-1} ethylenediaminetetraacetic acid disodium salt dihydrate), peroxide solution (0.08 mmol · L^{-1}) and succinate-lactate buffer (100 mmol · L^{-1}; pH 4.5) was used as described elsewhere (Baldrian, 2009). Samples were pipetted into the respective wells (four for each sample), and the substrate mixture was added. The plate was immediately placed in the reader, and the measurement was determined according to Štursová and Baldrian (2011), with slight modifications, such as extraction at room temperature.

The enzymatic activities of hydrolytic enzymes (1,4-β-D-glucosidase, cellobiohydrolase) were measured using the substrates 4-methylumbellyferyl-β-D-glucopyranoside (c = 2.75 mmol · L^{-1}) for β-D-glucosidase and 4-methylumbellyferyl-N-cellobiopyranoside (c = 2.50 mmol · L^{-1}) for cellobiohydrolase. Activities of the enzymes were measured as a change of fluorescence after 5 min and 125 min of incubating the microplates in an incubator (40 °C) with an excitation wavelength of 355 nm and an emission wavelength of 460 nm, according to Košnář et al. (2019).

Statistical analysis

The results are presented as the mean values of three replicates. Kruskal–Wallis analysis ($p \leq 0.05$), including testing for normality and homogeneity of data, and correlation analyses (0.05 level) were performed using STATISTICA 12 software (StatSoft, Tulsa, USA).

RESULTS AND DISCUSSION

Based on the results in Table 1, the water content of the substrate gradually decreased, which was most pronounced in the variant with 60% moisture (from 59.4% to 41.4%). During the colonisation phase, in most variants, higher water content values were measured in the upper layer of the substrate, whereas during fructification (from the primordia phase to the harvest phase), a higher water content value was found in the centre of the substrate. The lower water content in the upper layer of the substrate can be explained by the formation of primordia and their growth, which uses water from the substrate and transports it to the atmosphere. This finding is in agreement with the results of Atila (2019) for the cultivation of *Hericium erinaceus*. In this experiment, the author used different substrates such as oak sawdust, wheat straw or poplar sawdust with initial moisture levels of 68.5%–70%. During cultivation, the water content decreased by up to 17.3%. In our experiment, significant differences were found between the layers in the individual phases of cultivation, with the exception of the variant with a substrate water content of 70% and decrease was up to 27%. Furthermore, significant differences in water content values were found during the various stages of cultivation, especially between the colonisation of the substrate and the harvesting of the fruiting bodies. Bellettini et al. (2019) reported that water content in the substrate higher than 70% could cause development of diseases. However, in our case, the development of diseases and mould did not occur, even at a water content of 75%.

Another studied parameter of the substrate was pH. Based on the results, the pH value decreased during colonisation from 6 (before inoculation) to 4.74–5.43. This finding is in agreement with Bellettini et al. (2019), who reported that substrate pH is reduced, which is close to 4, during the colonisation stage. Obodai et al. (2003) used composted sawdust, banana leaves, maize stove and rice straw as substrates for cultivation, with initial pH values of 6.92–7.6 at a water content of 58%–70%. The pH values were higher than those observed in our study, most likely because of the different substrate composition. In our experiment, the pH value slightly increased from the colonization phase for all variants during cultivation. The lowest pH value (4.74) was measured for the variant of 60% water content in the substrate in the upper layer in the colonisation phase, and the highest pH value was found for the variant of 75% water content in the substrate in the harvest phase (5.83). In the colonisation phase, the pH values of all variants were higher in the middle layer of the substrate. In the primordial deployment phase, the pH of the upper layer was higher in the variant with 60% water content in the substrate; in the 65% and 70% water content variants, the pH was higher in the centre of the substrate. At the harvest stage, the pH in the centre layer was higher only for the 75% water content in the substrate. There was a statistically significant difference in pH values between the individual substrate layers, with the exception of the 70% water content in substrates, where no difference in the pH values was found, even in the individual phases of cultivation. For the 60%, 65% and 75% variants, statistically significant differences were found between the individual cultivation phases, especially between the colonisation and harvesting phases (Table 1).

The mycelium growth rates of *P. ostreatus* are presented in Figure 1. Variants with water contents of 65% and 75% showed the highest increase in mycelium growth per day. Between these two variants, there were no statistically significant differences. On the other hand, statistically significant differences were found between variants 60% and 70%, which also differed from the 65% and 75% variants. The variant with the lowest water content in the substrate had the lowest mycelium growth, which leads us to infer that an initial substrate water content of 60% is insufficient for adequate mycelium growth. Zervakis et al. (2001) tested growth rate of *P. ostreatus* and other mushrooms on different substrates with 65%–70% water content. Growth on wheat straw was 6.2 mm × day^{-1} which was slightly higher than that in our experiment (5.26 mm × day^{-1}). However, growth rate in our experiment was higher than that of *P. eryngii, P. pulmonarius* or *Lentinus edodes*, tested in their experiment.

Table 1. Changes of water content and pH in substrate during cultivation of *Pleurotus ostreatus*.

Parameter	Cultivation phase	60%		65%		70%		75%	
		1	2	1	2	1	2	1	2
Water content (%)	Colonisation	59.4 ± 0.7 aA	62.63 ± 0.76 aB	72.01 ± 1.09 aA	68.75 ± 1.07 aB	72.75 ± 1.89 aA	72.37 ± 0.53 aA	79.32 ± 0.69 aA	76.82 ± 0.66 aB
	Primordia	68.1 ± 0.87 aA	52.37 ± 1.02 bB	67.8 ± 0.65 bA	66.08 ± 0.63 bA	70.65 ± 1.21 abA	68.95 ± 0.19 bA	78.93 ± 1.06 aA	76.55 ± 0.37 aB
	First flush	41.4 ± 0.45 bA	57.7 ± 0.37 cB	58.5 ± 0.67 cA	66.55 ± 0.61 abB	68.17 ± 1.02 bA	67.28 ± 0.22 cA	74.55 ± 0.47 bA	75.0 ± 0.8 aA
pH	Colonisation	4.74 ± 0.03 aA	5.09 ± 0.01 aB	4.81 ± 0.02 aA	5.22 ± 0.01 aB	5.12 ± 0.12 aA	5.27 ± 0.06 aA	5.13 ± 0.07 aA	5.43 ± 0.04 aB
	Primordia	5.33 ± 0.19 bA	5.27 ± 0 abA	5.13 ± 0.01 bA	5.31 ± 0.03 bB	5.13 ± 0.06 aA	5.2 ± 0.01 aA	5.37 ± 0.06 bA	5.37 ± 0 aA
	First flush	5.29 ± 0.1 bA	5.35 ± 0.11 bA	5.5 ± 0.05 cA	5.63 ± 0.03 cB	5.16 ± 0.08 aA	5.26 ± 0.02 aA	5.83 ± 0.03 cA	5.63 ± 0.01 bB

Values are the means ± SD (n = 3).1 – upper layer of substrate, 2 – centre of substrate.
Different lowercase letters in a column indicate significant differences between stages of cultivation; capital letters indicate significant differences between layers of the substrate for each parameter (Kruskal–Wallis test, $p \leq 0.05$).

The BE is the ratio of the weight of the fresh fruiting body (g) per dry weight of substrate (g), expressed as a percentage (Liang et al., 2019). In our experiment (Figure 2), we found no statistically significant differences among the variants. However, the highest BE values (50% and 51%) were found for variants with 65% and 70% water content in the substrate, respectively. On the other hand, the lowest BE was found for the variant with 60% water content in the substrate, along with the lowest mycelium growth, indicating that this level of substrate moisture is not suitable for both adequate mycelium growth and high yields. In the experiment of Kalmış et al. (2008),

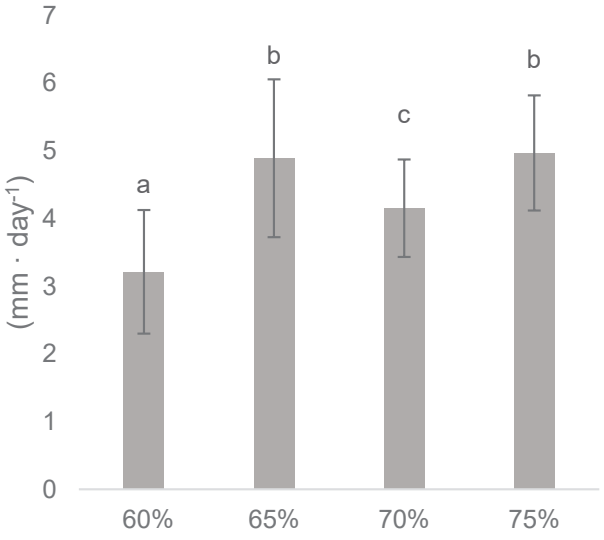

Figure 1. Mycelial growth rate at different substrate water content. Columns represent the mean values ± SD (n = 40). Different letters indicate significant differences (Kruskal–Wallis test, $p \leq 0.05$).

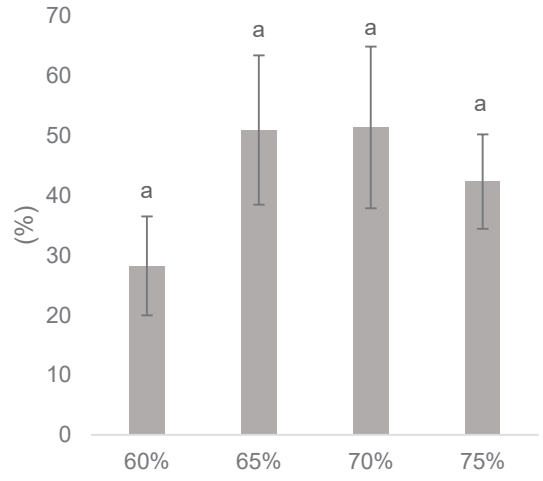

Figure 2. BE of substrate at different substrate water content. Columns represent the mean values ± SD (n = 3). Different letters indicate significant differences (Kruskal–Wallis test, $p \leq 0.05$). BE, biological efficiency.

Table 2. Changes of enzymatic activities in substrate during cultivation of *Pleurotus ostreatus*.

Enzymes	Cultivation phase	60%		65%		70%		75%	
		1	2	1	2	1	2	1	2
ß-D-G (µmol MUFG · h^{-1} · g^{-1})	Colonisation	474.88 ± 153.2 aA	221.65 ± 27.75 aA	275.17 ± 99.17 aA	560.2 ± 176.26 aA	717.26 ± 45.31 aA	508.59 ± 47.71 aB	184.77 ± 14.27 aA	260.08 ± 23.03 aB
	Primordia	252.16 ± 78.34 aA	219.69 ± 6.63 aA	1046.78 ± 10.52 bA	417.16 ± 18.37 aB	332.68 ± 34.07 bA	125.93 ± 25.27 bB	382.05 ± 35.24 aA	278.41 ± 91.8 aA
	First flush	236.59 ± 30.11 aA	570.39 ± 27.34 bB	843.88 ± 5.23 cA	691.2 ± 31.11 aB	678.78 ± 12.87 aA	290.46 ± 15.24 cB	3349.13 ± 549.5 bA	4083.42 ± 608.2 bA
Cell. (µmol MUFC · h^{-1} · g^{-1})	Colonisation	96.03 ± 9.69 aA	24.06 ± 4.03 aB	34.68 ± 6.18 aA	40.53 ± 12.42 aA	108.3 ± 28.03 aA	61.81 ± 4.08 aA	17.93 ± 11.54 aA	13.61 ± 5.07 aA
	Primordia	34.56 ± 19.44 bA	33.3 ± 15.28 aA	110.17 ± 27.99 bA	18.37 ± 8.37 aB	24.7 ± 2.61 bA	5.74 ± 0.3 bA	31.59 ± 7.79 aA	20.13 ± 5.9 aA
	First flush	30.74 ± 5.77 bA	59.15 ± 31.45 aA	85.59 ± 26.23 abA	71.47 ± 8.49 bA	110.23 ± 31.02 aA	25.57 ± 1.11 cB	931.88 ± 343.3 bA	1306.13 ± 387.54 bA
Mn – P (mU · g^{-1})	Colonisation	1.38 ± 0.01 aA	2.39 ± 0.91 aA	1.18 ± 0.54 aA	1.2 ± 0.64 aA	1.27 ± 0.09 aA	1.13 ± 0.42 aA	1.49 ± 0.09 aA	1.79 ± 0.13 aA
	Primordia	0.96 ± 0.37 aA	1.1 ± 0.03 aA	1.1 ± 0.3 aA	0.96 ± 0.12 aA	0.12 ± 0.13 aA	1.07 ± 0.24 aB	1.4 ± 0.17 aA	1.31 ± 0.04 bB
	First flush	0.09 ± 0.02 bA	1.55 ± 0.03 aB	0.92 ± 0.24 aA	0.62 ± 0.24 aA	0.92 ± 0.34 aA	0.55 ± 0.14 aA	0.76 ± 0.2 bA	0.53 ± 0.34 bA

Values are the means ± SD (n = 3). 1 – upper layer of substrate, 2 – centre of substrate.
Different lowercase letters in a column indicate significant differences between stages of cultivation; capital letters indicate significant differences between layers of the substrate for each parameter (Kruskal–Wallis test, $p \geq 0.05$).
MUFC, 4-methylumbellyferyl-N-cellobiopyranoside; MUFG, 4-methylumbellyferyl-ß-D-glucopyranoside.

P. ostreatus was cultivated on wheat straw mixed with olive mill effluent, and BE ranged from 14.7% to 50.7% at 70% water content in the substrate. Compared with our study, BE was lower for all substrate mixes Girmay et al. (2016) conducted experiments with different substrates. and BE of *P. ostreatus* cultivated on wheat straw (60%–70% water content in substrate) was 35.88%, which is higher than that in our experiment at a 60% water content and lower than our value for 65% and 70%.

The highest 1,4-ß-D-glucosidase activity (Table 2) was measured in the variant with a water content of 75% in the substrate at the harvest of fruiting bodies, when the activity increased over time in both substrate layers. Kannan et al. (1990) also found that the activity of 1,4-ß-D-glucosidase during the cultivation of *Pleurotus sajor-caju* increased over time. In their experiment, papermill substrate was used, with a cultivation period of 30 days. In our experiment, a statistically significant difference was found in this variant between the colonisation and primordial deployment phases and the harvest phase. This trend was also found for cellobiohydrolase activity at a water content of 75% in both layers, and at the same time, the highest values of cellobiohydrolase activity were measured (3,349.13 and 4,083.42 µmol · h^{-1} · g^{-1}, respectively). The values of 1,4-ß-D-glucosidase and cellobiohydrolase showed a strong correlation (correlation coefficient = 0.97), and the activities of these enzymes also correlated with the pH (correlation coefficients: 0.55 and 0.49). In the experiment of Velázquez-Cedeño et al. (2002), activity of cellobiohydrolase also increased during cultivation and was at its highest at the fruiting stage. The Mn-peroxidase values decreased during cultivation, and a negative correlation (-0.24 and -0.25) was found in connection with 1,4-ß-D-glucosidase and cellobiohydrolase (Table 2). This result is consistent with the findings of De Souza et al. (2006) who noted an increase in peroxidase activity within 10 days, followed by a decrease until 30 days of cultivation. Kurt and Buyukalaca (2010) cultivated *P. ostreatus* on different agricultural wastes. They reported the highest laccase activity on 10th day of cultivation, and then, activity slowly decreased. In our experiment, the laccase activities were below the detection limit.

CONCLUSIONS

The substrate moisture content decreased during cultivation, which can be attributed to development of *P. ostreatus* culture. The upper layer had a higher water content than the center of the substrate during primordia development and fructification because of the transport of water to the surface of the substrate for the growth of the fruiting bodies. The optimum substrate water content for mycelium growth and BE was 65%–75%, similar to previous studies. A lower water content is not suitable for proper mycelium growth and good yield. The activities of hydrolytic enzymes (1,4-ß-D-glucosidase and cellobiohydrolase) increased during cultivation,

which is connected to the gradual cellulose degradation. Variants with higher water contents in the substrate showed greater activities of these enzymes, which were correlated with the pH. The enzyme Mn–P is involved in lignin degradation, and its activity was higher in the early stage of cultivation. Differences in the activities in different substrate layers were significant mostly for variants with 65% and 70% water content, which also had the highest BE values. Consequently, for the cultivation of *P. ostreatus*, the most suitable substrate water contents range between 65% and 70%.

ACKNOWLEDGMENTS

The authors also thank Proof-Reading-Service.com Ltd., Devonshire Business Centre, Works Road, Letchworth Garden City SG6 1GJ, United Kingdom, for proofreading.

FUNDING

This research was funded by the Ministry of Agriculture of the Czech Republic (project QK1910235).

AUTHOR CONTRIBUTIONS

L.W. designed and performed experiments and analytical measurements, analysed data and wrote the paper. I.J. designed experiment. M.K. supervised experiment and provided funding, T.H. performed analytical measurements.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Atila, F. (2019). Lignocellulosic and proximate based compositional changes in substrates during cultivation of Hericium erinaceus mushroom. *Scientia Horticulturae*, *258*, 108779, doi: 10.1016/j.scienta.2019.108779.

Baldrian, P. (2009). Microbial enzyme-catalyzed processes in soils and their analysis. *Plant, Soil and Environment*, *55*(9), 370–378, doi: 10.17221/134/2009-PSE.

Baldrian, P., Valášková, V., Merhautová, V., and Gabriel, J. (2005). Degradation of lignocellulose by *Pleurotus ostreatus* in the presence of copper, manganese, lead and zinc. *Research in Microbiology*, *156*(5–6), 670–676, doi: 10.1016/j.resmic.2005.03.007.

Bellettini, M. B., Fiorda, F. A., Maieves, H. A., Teixeira, G. L., Ávila, S., Hornung, P. S., Júnior, A. M., and Ribani, R. H. (2019). Factors affecting mushroom *Pleurotus* spp. *Saudi Journal of Biological Sciences*, *26*(4), 633–646, doi: 10.1016/j.sjbs.2016.12.005.

De Souza, D. F., Tychanowicz, G. K., De Souza, C. G., and Peralta, R. M. (2006). Co-production of ligninolytic enzymes by *Pleurotus pulmonarius* on wheat bran solid state cultures. *Journal of Basic Microbiology*, *46*(2), 126–134, doi: 10.1002/jobm.200510014.

Dos Santos Bazanella, G. C., De Souza, D. F., Castoldi, R., Oliveira, R. F., Bracht, A., and Peralta, R. M. (2013). Production of laccase and manganese peroxidase by *Pleurotus pulmonarius* in solid-state cultures and application in dye decolorization. *Folia Microbiologica*, *58*(6), 641–647, doi: 10.1007/s12223-013-0253-7.

Elisashvili, V., Parlar, H., Kachlishvili, E., Chichua, D., Bakradze, M., Kokhreidze, N., and Kvesitadze, G. (2001). Ligninolytic activity of basidiomycetes grown under submerged and solid-state fermentation on plant raw material (sawdust of grapevine cuttings). *Advances in Food Sciences*, *23*, 117–123.

Girmay, Z., Gorems, W., Birhanu, G., and Zewdie, S. (2016). Growth and yield performance of *Pleurotus ostreatus* (Jacq. Fr.) Kumm (oyster mushroom) on different substrates. *AMB Express*, *6*(1), 87, doi: 10.1186/s13568-016-0265-1.

Goyal, M., and Soni, G. (2011). Production and characterization of cellulolytic enzymes by *Pleurotus florida*. *African Journal of Microbiology Research*, *5*, 1131–1136, doi: 10.5897/AJMR10.192.

Ihssen, J., Jankowska, D., Ramsauer, T., Reiss, R., Luchsinger, R., Wiesli, L., Schubert, M., Thöny-Meyer, L., and Faccio, G. (2017). Engineered *Bacillus pumilus* laccase-like multi-copper oxidase for enhanced oxidation of the lignin model compound guaiacol. *Protein Engineering, Design and Selection*, *30*(6), 449–453, doi: 10.1093/protein/gzx026.

Isikhuemhen, O. S., and Mikiashvilli, N. A. (2009). Lignocellulolytic enzyme activity, substrate utilization, and mushroom yield by *Pleurotus ostreatus* cultivated on substrate containing anaerobic digester solids. *Journal of Industrial Microbiology & Biotechnology*, *36*(11), 1353–1362, doi: 10.1007/s10295-009-0620-1.

Jin, L., Zeng, G., Chen, H., Wang, L., Ji, H., Lin, S., Peng, R., and Sun, D. (2021). Mechanism of lignin degradation via white rot fungi explored using spectral analysis and gas chromatography-mass spectrometry. *BioResources*, *16*(3), 5494–5507, doi: 10.15376/biores.16.3.5494-5507.

Kalmis, E., Azbar, N., Yildiz, H., and Kalyoncu, F. (2008). Feasibility of using olive mill effluent (OME) as a wetting agent during the cultivation of oyster mushroom, *Pleurotus ostreatus*, on wheat straw. *Bioresource Technology*, *99*(1), 164–169, doi: 10.1016/j.biortech.2006.11.042.

Kannan, K., Oblisami, G., and Loganathan, B. G. (1990). Enzymology of ligno-cellulose degradation by *Pleurotus sajor-caju* during growth on paper-mill sludge. *Biological Wastes*, *33*(1), 1–8, doi: 10.1016/0269-7483(90)90116-A.

Knop, D., Yarden, O., and Hadar, Y. (2015). The ligninolytic peroxidases in the genus *Pleurotus*: Divergence in activities, expression, and potential applications. *Applied Microbiology and Biotechnology*, *99*(3), 1025–1038, doi: 10.1007/s00253-014-6256-8.

Košnář, Z., Částková, T., Wiesnerová, L., Praus, L., Jablonský, I., Koudela, M., and Tlustoš, P. (2019). Comparing the removal of polycyclic aromatic hydrocarbons in soil after different bioremediation approaches in relationto the extracellular enzyme activities. *Journal of Environmental Sciences (China)*, *76*, 249–258, doi: 10.1016/j.jes.2018.05.007.

Kurt, S., and Buyukalaca, S. (2010). Yield performances and changes in enzyme activities of *Pleurotus* spp. (*P. ostreatus* and *P. sajor-caju*) cultivated on different agricultural wastes. *Bioresource Technology*, *101*(9), 3164–3169, doi: 10.1016/j.biortech.2009.12.011.

Liang, C.-H., Wu, C.-Y., Lu, P.-L., Kuo, Y.-C., and Liang, Z.-C. (2019). Biological efficiency and nutritional value of the culinary-medicinal mushroom *Auricularia* cultivated on a sawdust basal substrate supplement with different proportions of grass plants. *Saudi Journal of Biological Sciences*, *26*(2), 263–269, doi: 10.1016/j.sjbs.2016.10.017.

Maciel, G. M., Bracht, A., Souza, C. G. M., Costa, A., and Peralta, R. (2012). Fundamentals, diversity and application of white-rot fungi. In A. Paz-Silva and María Sol (Ed.), *Fungi: Types, environmental impact and role in disease* (pp. 409–458). Hauppauge, New York, USA: Nova Science Publishers, Inc.

Mikiashvili, N. A., Chichua, D., and Elisashvili, V. I. (2004). Lignocellulolytic enzyme activities of medicinally important Basidiomycetes from different ecological niches. *International Journal of Medicinal Mushrooms*, *6*(1), 1–10, doi: 10.1615/IntJMedMushr.v6.i1.70.

Mikiashvili, N., Wasser, S. P., Nevo, E., and Elisashvili, V. (2006). Effects of carbon and nitrogen sources on *Pleurotus ostreatus* ligninolytic enzyme activity. *World Journal of Microbiology and Biotechnology*, *22*(9), 999–1002, doi: 10.1007/s11274-006-9132-6.

Obodai, M., Cleland-Okine, J., and Vowotor, K. A. (2003). Comparative study on the growth and yield of *Pleurotus ostreatus* mushroom on different lignocellulosic by-products. *Journal of Industrial Microbiology & Biotechnology*, *30*(3), 146–149, doi: 10.1007/s10295-002-0021-1.

Pánek, M., Wiesnerová, L., Jablonský, I., Novotný, D., and Tomšovský, M. (2019). What is cultivated oyster mushroom? Phylogenetic and physiological study of *Pleurotus ostreatus* and related taxa. *Mycological Progress*, *18*(9), 1173–1186, doi: 10.1007/s11557-019-01515-0.

Siwulski, M., Sobieralski, K., and Miran, D. (2007). The effect of moisture of the substrate on yielding of two oyster mushroom strains. *Roczniki Akademii Rolniczej w Poznaniu, Ogrodnictwo, 41383*, 615–618 (in Polish).

Šťursová, M., and Baldrian, P. (2011). Effects of soil properties and management on the activity of soil organic matter transforming enzymes and the quantification of soil-bound and free activity. *Plant and Soil*, *338*(1), 99–110, doi: 10.1007/s11104-010-0296-3.

Velázquez-Cedeño, M., Mata, G., and Savoie, J.-M. (2002). Waste-reducing cultivation of *Pleurotus ostreatus* and *Pleurotus pulmonarius* on coffee pulp: Changes in the production of some lignocellulolytic enzymes. *World Journal of Microbiology and Biotechnology*, *18*, 201–207, doi: 10.1023/A:1014999616381.

Wang, D., Sakoda, A., and Suzuki, M. (2001). Biological efficiency and nutritional value of *Pleurotus ostreatus* cultivated on spent beer grain. *Bioresource Technology*, *78*(3), 293–300, doi: 10.1016/S0960-8524(01)00002-5.

Zervakis, G., Philippoussis, A., Ioannidou, S., and Diamantopoulou, P. (2001). Mycelium growth kinetics and optimal temperature conditions for the cultivation of edible mushroom species on lignocellulosic substrates. *Folia Microbiologica*, *46*(3), 231–234, doi: 10.1007/BF02818539.

Received: October 31, 2022; accepted: January 2, 2023