Wood decomposition activity of oyster mushroom (*Pleurotus ostreatus*) isolate in situ

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ABSTRACT: The purpose of the research was to examine the decomposition rate of beech (*Fagus sylvatica*) and aspen (*Populus tremula*) logs after an oyster mushroom (*Pleurotus ostreatus*) infection as well as the effect of fruit body formation on the chemical composition of wood. The highest mean value of biological efficiency (B.E.) was found on beech logs (29.05%), but relatively high values of B.E. were recorded also on aspen logs (21.69%). The average content of N was about 37% higher in inoculated logs than in the control (not inoculated) ones. The ash content was about 90% higher in inoculated logs. The content of the mineral elements Ca, Mn, Zn, Cu, Pb, Al, B, and Mg had an increasing tendency in inoculated logs compared with control ones. The logs with minimal production of fruit bodies had the highest content of the mineral elements Pb, Al, Fe, Mg and P.

Keywords: oyster mushroom; *Pleurotus ostreatus*; fruit body production; decomposition rate; content of mineral elements; biological efficiency

Fungi are an intrinsic part of forest ecosystems and a critical constituent for their existence and restoration. A great diversity and diverse ecological functions of the fungi species in nature are the basis of ecological stability in the forest communities. Permanently greater attention has recently been paid to their participation in forest development, silviculture, forest regeneration, and forest protection.

The genus *Pleurotus* (oyster mushrooms) comprises some of the most popular edible mushrooms due to their favourable organoleptic and medicinal properties, vigorous growth and undemanding cultivation conditions (Gregori et al. 2007). The oyster mushroom is a common saprophytic fungus in Slovak forests. Although it is commonly growing on dying trees, it behaves as a facultative parasite at the earliest opportunity (Stamets 2000). From the viewpoint of forestry, it is primarily a saprophytic fungus speeding up wood decomposition. This ability could be used for biodegradation of waste wood unserviceable in forest management or wood technology. This ability can be used for speeding up wood decomposition in more extreme conditions, mainly at rocky sites and areas with steep inclines and where the wood is not processed and it serves for soil enrichment of poor sites. It is the basis for soil formation as well. Very important is the ability of the oyster mushroom to filter pollutants from soil, and to strengthen unstable and eroded sites. The ability of this fungus to degrade wood cell walls and carry out the cooperative processes with other fungi and bacteria plays a key role in the successful decomposition of organic substances, formation of simple organic products, establishment of the soil community and formation of fertile soil (Heilmann-Clausen, Boddy 2005; Piškur et al. 2009, 2011).

White rot fungi are currently extensively studied for bioremediation purposes (Gao et al. 2010). Possibilities for practical utilization of the oyster mushroom in Slovak forest management are extensive and the results of its utilization are well-known as well. During research carried out in the 1980s,

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the oyster mushroom growth and fruit body production were examined on wood of various tree species (Pavlík 2005). The requirement to solve the processing of waste dendromass by ecological near to nature methods as well as new information on the abilities of the oyster mushroom were the main reasons for the new research. Our attention was focussed not only on fruit body production but also on both the decomposition rate and changes in the chemical composition of European beech (*Fagus sylvatica)* and European aspen *(Populus tremula)* wood due to the oyster mushroom activity. There are two main reasons for our investigations: (1) a need for testing possible ways of using wood-destroying fungi for effective decomposition of waste dendromass under natural conditions in a forest stand, and (2) an examination of relationships between fruit body production and changes in the chemical composition of wood.

MATERIAL AND METHODS

The research was carried out in the Borova hora Arboretum – a research and development workplace of the Technical University in Zvolen (Slovak Republic) during 2006–2011. During five years of research, the process of fruit body formation was analysed in detail on 44 aspen and 40 beech logs.

Pleurotus ostreatus var columbinus isolate MT-FCCA019 was obtained from the Mykoforest Type Culture Collection of the Mykoforest Company in Velcice, Slovakia. Primarily it was isolated from sporocarps growing on a dead trunk of *Fagus sylvatica* in a forest stand 407 m a.s.l. in December 2003, in the Tribec Mountains, Slovakia. It is a natural strain, therefore it is suitable for overgrowing on the beech and walnut tree and poplar wood directly in forest stands.

Logs 30–40 cm in diameter and 50 cm long were inoculated with the oyster mushroom inoculum in the form of grain spawn. A new method of intensive inoculation using a special applicator – the mushroom funnel was developed during the period of research. According to this method, the inoculum is pressed into longitudinal, 2–3 cm deep snicks at the sides of logs, and subsequently is painted with protective coating. Inoculation is practically without loss of grain spawn, latex coating is relatively cheap, and application is very easy, without any undesirable effects on mushroom growth. The inoculated logs were then wrapped in a plastic cling-film and placed in the shadow below the canopy of mature forest stand for approximately three months.

The logs were unwrapped after the first rainy and foggy days. They were dug into the soil to a depth of 10–15 cm at the same place. No other maintenance or protection of logs was undertaken, and they were exposed to the activity of oyster mushroom and other wood-destroying fungi, as well as various biotic and abiotic factors. A part of logs was not inoculated with the oyster mushroom (control sample).

During the five-year production cycle we evaluated the fruit body production, biological efficiency of wood decomposition and the chemical composition of wood from selected beech and aspen logs inoculated and not inoculated by the oyster mushroom. The biological efficiency of decomposition, as the ability of mushrooms to convert substrate materials into fruit bodies, was measured using a simple formula known as the biological efficiency formula (B.E.). This formula states that one pound of fresh mushrooms grown from one pound of dry substrate is 100% biological efficiency (Stamets 2000). The biological efficiency was calculated based on actual weight and humidity of logs at the beginning of the experiment, and actual weight of fresh fruit bodies growing up on the logs during five years. The humidity of fresh beech wood was 30%, that of aspen wood 50%.

Chemical analyses of wood after termination of the oyster mushroom fruit body production were done in the Forestry Sciences Laboratory (National Forestry Centre in Zvolen). Samples of homogenised wood mass were taken from logs with the highest values of B.E., from logs with the lowest B.E values, and from control logs of both tree species.

The samples were dried up using the APT.line FED Series dryer, a product of the BINDER Company, at a temperature of 60°C until the constant weight of wood samples. Then the samples were homogenized using the SM-100 cutting mill (Retsch Co., Haan, Germany). The microwave disintegration in 5% HNO_3 was done by the MARSXpress device. The content of C and N was analysed with ThermoFinnigen Flash 1112 by dry burning in the flow of O_2 (at 1,250°C), the ash content by burning in the muffle furnace at 900°C, the content of Pb by atomic absorption spectrophotometry using the Hermo iCE 3000 Series device, and the contents of all other mineral elements were analysed by ICP atomic emission spectrophotometry using the Varian 725-ES device.

Statistical significance of the differences in the content of particular macro- and microelements in the wood of control logs and logs with maximal and minimal production of fruit bodies was tested, owing to the small sample size, by the nonparametric Kruskal-Wallis median ANOVA test using the STATISTICA 7.0.

RESULTS

Fruit body production

During the research period, fruit bodies of the oyster mushroom were formed on all inoculated logs. The most productive beech log produced 12,560 g and the most productive aspen one 10,640 g of fruit bodies during five years (Table 1).

Comparing the quantity of fruit body production, beech is the better tree species for the oyster mushroom production. Total yield of fruit bodies from 40 beech logs was 353,695 g, more than twice higher compared with the total production on 44 aspen logs (157,100 g). Maximal production was reached in the third year, but the second year was very good for production as well. In the fourth and especially in the fifth year the production of fruit bodies was very low (Table 1). The control logs were without oyster mushroom fruit bodies.

The mean value of B.E. during the research period was 29.05% on beech logs and 21.69% on aspen logs. Maximal values of B.E. on particular logs reached 47.75% on beech logs and 45.10% on aspen ones. Minimal values of B.E. were 7.79% on aspen and 17.51% on beech logs (Table 1).

Chemical analyses of logs after a period of fruit body production

Generally, the activity of the oyster mushroom did not have a significant impact on the content of C in logs. The content of C in the control log and in the one with maximal or minimal B.E. values was approximately the same both in the aspen and beech logs (Table 2).

A distinct difference was found in the content of N. Inoculated logs had on average about 37% higher content of N than control ones. An increase in the ash content by approximately 90% in inoculated logs was evident as well. The content of the mineral elements Ca, Mn, Zn, Cu, Pb, Al, B, and Mg showed an increased tendency in inoculated logs. The highest increase was found in Al (280% on average) and the lowest one in Zn (50% on average). The content of Fe, Na and P was mostly higher in inoculated logs, but its decrease was also found in inoculated logs compared with control ones. The only element with a significant decrease of its content in inoculated logs was K. The extremely high content of K in aspen logs is an exception. The content of N and mineral elements was increasing more intensively in beech logs except for Al, the content of which was higher in the inoculated aspen logs (Table 2).

Fruit body production has a specific impact on the chemical composition of wood. The logs with minimal production of fruit bodies and the logs with the lowest value of B.E. had the highest content of mineral elements Pb, Al, Fe, Mg and P. The beech and aspen logs with the highest value of B.E. had the highest content of Zn (Table 2).

The effect of the intensity of fruit body formation on the chemical composition of wood was not always the same. For example, the content of mineral elements Ca, Mn, Cu, B, and Na was higher in aspen logs

Table 1. Mean, maximal and minimal values of fruit body yields and biological efficiency (B.E.) on aspen and beech logs during the years 2006–2010

Tree species	Parameter	Stumps			Fruit body production (g)					Total	
		thickness (cm)	length (cm)	dry substrate (g)	2006	2007	2008	2009	2010	yield (g)	B.E. (%)
Aspen	mean	28	50	16,460	483	1,344	1,488	245	10	3,570	21.69
	total			724,290	21,250	59,145	65,470	10,795	440	157,100	
	max. B.E.	34	50	23,590	0	2,380	5,800	2,360	100	10,640	45.10
	min. B.E.	34	46	21,710	500	410	480	300	0	1,690	7.79
	max. prod.	34	50	23,590	0	2,380	5,800	2,360	100	10,640	45.10
	min. prod.	33	44	19,560	320	870	450	0	0	1,640	8.38
Beech	mean	33	51	30,440	819	2,856	4,548	605	14	8,842	29.05
	total			1,217,410	32,760	114,255	181,930	24,180	570	353,695	
	max. B.E.	29	46	20,350	900	3,335	5,270	210	0	9,715	47.75
	min. B.E.	35	46	29,640	0	1,720	2,810	660	0	5,190	17.51
	max. prod.	36	54	36,810	1,760	2,960	7,300	540	0	12,560	34.12
	min. prod.	22	50	12,730	270	1,485	1,570	480	0	3,805	29.89

Table 2. Mean content of wood elements in control logs and in logs with maximal and minimal biological efficiency (B.E.) values

Wood element		Control logs		Logs with B.E. values			
				minimal		maximal	
		aspen	beech	aspen	beech	aspen	beech
Dry matter	(%)	96.41	96.63	97.68	95.87	97.75	96.19
Ash	(%)	1.89	1.16	3.46	2.27	3.47	1.16
Total C	(%)	49.2	49.6	48.6	49.6	50.3	48.5
Total N	(%)	0.32	0.2	0.36	0.35	0.45	0.24
Total Ca	(mg·kg^{-1})	4,474	2,429	7,653	6,753	8,317	13,071
Total Mn	(mg·kg^{-1})	35.9	127.5	60.5	360.7	61	228.8
Total Zn	(mg·kg^{-1})	48.5	10.4	79	16.6	63.3	15.2
Total Cu	(mg·kg^{-1})	6.68	5.74	10.77	17.88	11.08	11.9
Total Pb	(mg·kg^{-1})	1.67	1.35	2.4	1.98	4.08	3.78
Total Al	(mg·kg^{-1})	91.9	78.5	250	107.6	521.7	431.6
Total B	(mg·kg^{-1})	4.51	4.04	6.99	8.15	7.32	6.2
Total Fe	(mg·kg^{-1})	307.2	137.4	412.3	129.6	463	443.7
Total K	(mg·kg^{-1})	4,276	2 856	1,130	1,862	8,296	1,005
Total Mg	(mg·kg^{-1})	517.8	424.5	580.8	706.3	982.3	905.3
Total Na	(mg·kg^{-1})	56.2	38.1	47.7	61.2	108.6	55.8
Total P	(mg·kg^{-1})	415.3	222.5	317.3	300	627.8	343.3

with the lowest values of B.E., but it was highest in beech logs with the highest values of B.E. (Table 2).

In beech and aspen logs, the differences in the content of dry matter, ash, N, C, Ca and Fe were not statistically significant (Kruskal-Wallis test, $P > 0.05$). Differences in the content of Zn, Pb, Al, Mg and Na in beech logs, and Mn, Cu and B in aspen logs were not statistically significant either.

The content of Mn, Cu and B was statistically significantly (Kruskal-Wallis test, $P < 0.05$) higher in inoculated beech logs compared with control ones. In aspen, the content of Zn, Pb, Al, Mg and Na was statistically significantly higher in inoculated logs with minimal production of fruit bodies compared with both the inoculated logs with maximal production and the control ones (Kruskal-Wallis test, $P < 0.05$). The content of K was statistically significantly lower in inoculated beech logs compared with control ones (Kruskal-Wallis test, $P < 0.05$). In aspen, the highest content of K was in logs with maximal production, lower in control ones, and the lowest in logs with minimal production of fruit bodies (all differences were statistically significant, Kruskal-Wallis test, $P < 0.05$).

DISCUSSION

Pleurotus spp. cultivation is considered to be a very simple procedure in the case of log cultivation because it does not involve any sophisticated equipment. However, despite its simplicity, large-scale cultivation on natural logs is not often used due to long incubation periods, low yields and environment-dependent production if conducted outdoors (Gregori et al. 2007). The white rot fungus *Pleurotus ostreatus* (Jacq.) P. Kumm. is one of the most successful wood and soil colonizers and has been frequently used for bioremediation purposes (Baldrian 2008).

Broadleaf hardwood is a natural substratum for oyster mushroom growing. There is a direct relationship between the quality and other attributes of oyster mushroom fruit bodies and the quality of the substrate wood. Almost 30% biological efficiency of wood decomposition reached in our experiment after five years of cultivation is a very good result for natural, non-sterile conditions in forestry practice, and moreover, with economic profit, although the quantity of fruit bodies was not our priority. Maximal production was reached in the third year, but the second year was very good for production as well. In the fourth and especially in the fifth year the production of fruit bodies was very low (Table 1). This indicates a very intensive phase of substrate decomposition, and therefore the production of fruit bodies is already under economic efficiency and practically uninteresting. More important is that we have confirmed the suitability of the oyster mushroom for natural decomposition of waste wood unserviceable in forest management or wood technology.

The content of mineral components in wood such as Ca, Mg, P, Si, K and others is normally low, and made up mainly of oxides. The ash content in wood is also considered low, varying between 0.2 and 1% of dry weight (Browing 1963). These results were confirmed in our experiment except for K, the content of which increased only in aspen logs with minimal production of fruit bodies.

According to Kurtzmann and Zadražil (1982), P, K, Fe and Mg are the most important minerals for the cultivation of the oyster mushroom. Sales-Campos et al. (2009) confirmed in their study that these elements are naturally present in all the raw materials used for the preparation of the cultivation substrate. They found that the substrate during cultivation underwent a modification, resulting in a different substrate composition from that of the initial one. According to Oliveira (2000), besides physical, environmental, chemical and biological factors, the degradation level also varies with the genetic composition of the *Pleurotus* species used.

Fe, Zn, Al, Mn, Cu, Cr and Mo are among the most studied and most essential micronutrients (trace elements) for the growth of many species of fungi (Miles, Chang 1997). Some chemical elements that have been detected in the constitution of fungi, however, do not necessarily indicate any biological importance. It is hard to determine experimentally the necessary amount of these elements because the tested element may be present in sufficient amounts, in impure form, in an ingredient of the growth medium or may be introduced by means of the inoculum. These elements are constituents or activators of several enzymes (Miles, Chang 1997).

Zadražil (1978) analysed the content of mineral elements in the different developmental stages of *Pleurotus ostreatus* and found increasing content of N, P, K, Ca, Mg and ash in the substrate until the stages before fruiting, followed by a slight decrease in N, K and P contents showing a selective removal of nutrients towards the basidioma in the process of fruit body formation. Nevertheless, the spent substrate showed an increase of mineral element content compared with the initial one. The author reported that the spent substrate, rich in nutrients, showed high digestibility due to cellulose and lignin degradation, with increased solubility, consequently offering the higher content of free sugars (glucose), which makes it useful as a basis for the champignon compost, organic fertilizer and animal feed. Sales-Campos et al. (2009) found a remarkable increase in minerals contents in the substrate decomposed by the fungus as well.

The process of increasing the content of N, P, K and Ca was completely confirmed also by our results. It is evidenced by the lowest content of those elements in control logs, their maximal content in logs with the minimal production of fruit bodies (minimal B.E.), and the decrease of their content in logs with the maximal fruit body production (maximal B.E.).

The relative increase of the mineral content in spent substrates was also verified in other studies (Sturion 1994; Oliveira 2000; Silva et al. 2002), resulting from the cultivation of different *Pleurotus* strains in several agricultural residues. Ca was the element with the highest content in a spent substrate in the cited studies and also in our experiment.

Generally, the activity of the oyster mushroom does not have a significant impact on the content of C in logs. The content of C in the control logs and in those with maximal or minimal B.E. was approximately the same both in the aspen and beech logs. This suggests that the oyster mushroom growing on wood logs is not an obstacle for sequestration of carbon.

Results of our experiment confirmed the majority of the results of similar researches, although most of them were focussed on substrates made from a mixture of various wood and plant materials. The results presented here confirmed a manifold increase in the rate of waste wood decomposition by the oyster mushroom activity. The possibility of successful application, continual growing, and fruit body production of the oyster mushroom under natural, not sterile conditions was also confirmed. That is very important because of the aims of the research – effective, ecological, and rapid decomposition of the waste wood under forestry conditions.

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