CULTIVATION OF *PLEUROTUS OSTREATUS* MUSHROOM ON WASTE PRODUCTS AND COMPOST FOR PHENOL DEGRADATION

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Abstract. Remediation through application of different kinds of mushrooms is relatively limited due to different objective factors affecting the growth and development of the mushroom species. This paper presents the results of experiments performed to cultivate the *Pleurotus ostreatus* mushroom on substrates based on various organic wastes and composts. The mushroom mycelium blocks were transferred to soil mixtures and the period of adaptation and sporophores formation was monitored. From sporophores was isolated and purified enzyme tyrosinase, which was tested for phenol degradation activity. The obtained results, show's that the dense homogeneous blocks formed between the mycelium and the substrate can be used for the recovery of solid waste products for direct application to soils contaminated with phenol.

Key words: *Pleurotus ostreatus*, bioremediation, toxic compounds degradation, phenol decomposition, compost.

INTRODUCTION

The use of mushrooms as a method of bioremediation provides opportunities to clean up pollutants from the environment, primarily in soils. *Pleurotus ostreatus* (*P. ostreatus*) mushrooms are disease resistant and their cultivation does not require sophisticated control. These mushrooms are easily cultivated on a variety of nutritive substrates, including some waste products [1]. Girmay et al. [2] have reported that *P. ostreatus* mushrooms can grow on cottonseeds, paper wastes, wood saw-dust and wheat straw, with varying growth rates. The highest biological and economic yields, as well as the highest percentage of bio-efficiency of the mushrooms studied, were obtained using cotton seeds, while the lowest was the wood saw-dust.

The mycelium of *Pleurotus ostreatus* produce enzymes and acids degrading lignin and cellulose, which structurally resemble a variety of organic contaminants. Lignolytic mushrooms are used for the removal of aromatic pollutants and hard degradable chlorinated contaminants [3, 4]. Increasing studies indicate that mushrooms can transform many structures of pharmaceutical compounds, including anti-inflammatories, β -blockers, and antibiotics [5]. They have the ability to grow on different crude lignocellulosic substrates and over a wide range of temperatures [1]. For example the results, published from Ritota and Manzi [6] show that the mushrooms from genus *Pleurotus* are suitable for cultivation both in different climates and on a variety of substrates, including conifer wood and even pine needles.

There are also a number of benefits from growing *Pleurotus ostreatus*: utilizing the waste generated and purifying the available organic pollutants; use of the sponge for feeding animals and for extracting enzymes and medicinal compounds [7, 8].

In the transition to a circular economy of modern society, the focus has shifted from a vision

to the creation of real policies. Under this new concept, different types of waste, including agricultural waste and household waste streams, are considered a valuable resource. Mushroom cultivation on various waste-based substrates can be sustainable economically and environmentally. This would lead not only to the production of edible and/or medicinal mushrooms, but also to a spent mushroom substrate (SMS), which can find a wide variety of applications. Fig. 1 shows the use of SMS in a circular economy according to [7].

Kh.Alananbeh et al. [9] reported on study conducted in 2014 on mushroom cultivation upon palm leaf waste mixed with other agro-waste generated. In this study, the agricultural waste used are mixed with palm leaves in different proportions with two types of additives, and the mixture from wheat straw and date palm in a 3:1 ratio shows the best results.

Phenol is a highly toxic compound that has a high degree of bioaccumulation along the food chain and has a proven negative impact on living organisms and the environment. For example, its effect on aquatic organisms is manifested even at relatively low concentrations in the range of 5-25 mg / L [10]. Therefore, its removal or neutralization into the environment poses a real challenge for researchers.

In our previous investigation, the coffee sludge as a nutrient substrate for the cultivation of *Pleurotus ostreatus* was used, and it was found that the mycelium was successfully developing in heavy metal contaminated soil. The efficacy of *P. ostreatus* in the absorption of Cu, Zn, Ni, Al, Bi, Mn and Fe from the soil has been demonstrated [11].

No published data were found for the cultivation of fungal mycelium in contaminated soils taken from the natural environment (not created under laboratory conditions). In addition to the inclusion of various organic composts in the raw material cycle, the

possibility of developing micellar blocks in such real contaminated soils has been explored in order to improve their properties, cleanse them from pollutants and restore equilibrium.

The purpose of current work is to cultivate a mushroom (*Pleurotus ostreatus*) on substrates based on organic waste and compost, with the application of direct degradation of phenol in water and soil.

Fig. 1. Use of SMS in a circular economy [7].

MATERIALS AND METHODS

The experimental work consists three main stages:

1. Cultivation of *Pleurotus ostreatus* in five different substrates to form a homogeneous block of micelles

The mushroom culture was prepared according to [6] on malt extract agar medium for 7 days. Fifty gram of malt extract agar was mixed with 1L of distilled water. Test tubes were corked, sterilized (at 121 °C, for 30 min). To obtain pure culture, a piece (5 mm×5 mm) of fleshy tissue was aseptically transferred to individual malt extract agar slants under UV fitted inoculation chamber. The cultures were incubated at 25 °C until sufficient mycelia growth. The slant culture was transferred to petri-

plates and incubated at 25 °C for 7 days. Pieces of agar (1x1 cm) with mycelium were transferred directly to the surface of the test substrates, evenly distributed over their surface. The content of substrate forming materials is described in detail in Table. 1. Samples were sterilized at 121 °C in an autoclave for 20 min and then cooled to room temperature. They are all placed in plastic containers of 1L capacity.

The moisture of the substrate used for cultivation should be 70-80% and the sterilized mixture was inoculated with previously grown pure cultures of the *Pleurotus ostreatus* strain. The inoculated samples were incubated in the dark at room temperature (26-28°C) until complete colonization of substrates from the mycelium.

Table 1. Raw materials used for *Pleurotus ostreatus* cultivation

№	Raw materials - substrates (150 g)	Supplements (15g)
1	Compost from hardwood	perlite
2	Compost from conifer wood	perlite
3	Compost from municipal solid wastes	perlite
4	Coffee grounds	perlite
5	Hardwood saw-dust	perlite
6	Hardwood saw-dust	no

2. Laying the mycelial block in containers with soil mixtures to monitor the adaptation of the mycelium in the soil environment

For the purposes of the study, a sample of soil material was taken from a material heap at a mine for the extraction of non-ferrous metals. This soil material is characterized by a lack of organic component, low nutrient reserve (N, P, K, Ca, Mg, Na, S) and increased concentrations of heavy metals

(Cu, Zn, Ni, Al, Bi, Mn and Fe). The soil material samples were pre-treated by drying, removing rock fragments and large rock specimens, crushing and sieving (2 mm), then preparing three mixtures with coffee sludge additives, CaCl_2 and $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$. Used for comparison and a clean sample of soil material (no additives). The resulting mycelial blocks were transferred to the pre-prepared mixtures, the composition of which is given in Table 2.

Table 2. Soil mixtures used for experiments

№	Soil samples	amount (g)
1	Soil	450
2	Soil and coffee grounds (1:1)	450
3	Soil and CaCl_2	400 + 50
4	Soil and $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	400 + 50

The period of adaptation of the mycelial blocks involved in the mixtures and the time of sporophores formation were monitored.

3. Isolation of tyrosinase enzyme from sporophores formed and degradation of phenol as model substrate

The isolation and purification of the tyrosinase enzyme from the sporophores of *Pleurotus ostreatus* was performed according to the methodology described in [12]. The isolation and purification process are shown schematically in the figure below.

Fig. 2. Steps for isolation and purification of tyrosinase from mushroom's sporophores

Isolated and purified tyrosinase was assayed for enzymatic activity for phenol degradation. The procedure was performed as described in [13].

The reagents used in the experimental work at this stage and their quantities are given in Table 3.

Table 3. Reagents used for phenol degradation procedure

№	reactive	control sample	isolated tyrosinase
1	phosphate buffer 0.5 M KH_2PO_4 , 0.6 M K_2HPO_4 (pH=7.9)	65 μl	65 μl
2	0.5 M NH_4OH	75 μl	75 μl
3	100 mM 4-aminoantipyrine	30 μl	30 μl
4	250 mM potassium ferricyanide	30 μl	30 μl
5	phenol in d. water	2800 μl	2800 μl
6	enzyme solution	100 μl d water	100 μl

Phenol with concentrations of 1.2 $\mu\text{g/ml}$, 1.6 $\mu\text{g/ml}$ and 2.0 $\mu\text{g/ml}$ for a period of 72 hours was used for the experiment. The measurements were performed with a spectrophotometer VWR634-6001, UV-1600PC (Germany) at a wavelength $\lambda = 500 \text{ nm}$.

The rate of degradation of phenol in wastewater was monitored and samples were photometrically determined at intervals after plating, at 1, 3, 6, 12, 18, 24, and 72 hours.

RESULTS AND DISCUSSION

In the present *Pleurotus ostreatus* cultivation experiment, different substrates were used: deciduous wood compost, coniferous wood compost, solid waste compost, coffee sludge, and deciduous wood chips. Although in its natural environment, *Pleurotus ostreatus* grows on deciduous wood, the species has proven to be adaptable to a variety of substrates, including coniferous compost [14]. The coniferous wood compost used, nonspecific as the

natural environment of *P. ostreatus* development, is the probable reason, despite the formation of a stable mycelial block in the starting substrates, to not observe the appearance of sporophores after its transfer to soil mixtures.

Cultivation of *P. ostreatus* based on waste coffee residues (WCR) from coffee consumption is a new way of recycling this waste product. The presence of caffeine inhibits the growth of micelles on agar and in liquid culture in the laboratory. Carrasco-Cabrera reported that elevated WCR levels in the WCR/sawdust substrate also slow the growth of the mycelium and delay or prevent fruiting during commercial cultivation. The timing of mycelium formation in the present experiment coincides with that reported in [15] - 18 days.

Table 4 presents the time data for the formation of micelles on different nutrient media. The same table shows the time for the formation of sporophores after transferring the mycelial block to soil mixtures.

Table 4. Time for colonization and fruiting (in days)

№	Raw materials (substrates)	Complete colonization of the substrate	Fruiting after transfer of the mycelium into the soil mixtures
1	Compost from hardwood	17	14
2	Compost from conifer wood	25	no fruiting
3	Compost from municipal solid wastes	25	14
4	Coffee grounds (waste coffee residues)	18	12
5	Hardwood saw-dust	20	15
6	Hardwood saw-dust without perlite	22	16

The cultivation practice of *P. ostreatus* shows that 2-3 mushroom harvests are harvested, after which the spent nutrient substrate is discarded. Koshy et al reported that this substrate can be used effectively to remove phenol from wastewater up to 48% after 1 day of treatment. The process guarantees the double benefit of the growing – production of mushrooms with nutritional/healing properties and using the spent substrate for bioremediation. The process also offers a sustainable solution for the management of solid wastes from spent mushroom substrate [10].

In this regard, the collected sporophores of *Pleurotus ostreatus* were used to isolate and purify tyrosinase and were applied to the subsequent

degradation of phenol. The results presented in Fig. 3 show that at the twelfth hour of treatment, a 42% degradation rate of phenol was observed, at the twenty-fourth hour - 58%, and at the seventy-second hour, more than 71% degradation was achieved in the sample with concentration 1.2 µg/ml. At higher concentrations of phenol (1.6 µg/ml and 2.0 µg/ml), its degradation rate does not exceed 40-42% and remains almost constant for the time between the twenty-fourth and seventy-second hours. The likely reason for this is the high concentration of pollutants in the soil that exert an inhibitory effect on enzyme activity. On Fig. 3 the correlation between the degraded phenols versus time in different phenol concentrations are shown.

Fig. 3. Correlation between the percentages degraded phenol versus time. Phenol concentration 1.2 µg/ml, 1.6 µg/ml and 2.0 µg/ml.

The results obtained correspond to those reported in [16], where a phenolic degradation rate of 100% was achieved under similar experimental conditions. The probable reason for the lower percentage of degraded phenol in the experiment was that the mycelial blocks had previously undergone an adaptation step in soil samples, which reduced the enzymatic activity in the fungi.

There are numerous studies using micro-remediation at artificially polluted organic pollutants, but the significance of studies under non-sterile conditions, with real soil/wastewater contamination, and results can be transferred to the field using

biostimulation, bioaggregation or both [17]. In this regard, the experimental results of the study showed the possibility of cultivating *P. ostreatus* mushroom fungus in soil material taken from the environment containing elevated concentrations of heavy metals, as well as growth the mycelium in such an environment in order to improve the properties and purification from pollutants.

CONCLUSIONS

Based on the experiments performed, the following conclusions can be summarized. *Pleurotus ostreatus* mycelium was successfully cultivated on five

different food substrates: compost from hardwood, compost from conifer wood, compost from municipality solid wastes, coffee sludge (waste coffee residues) and hardwood saw-dust.

The formed blocks of mushroom micelles were successfully introduced into mixtures based on soil material characterized by low fertility, poor nutritional content and high content of heavy metals, with fruiting times between 12 and 16 days (except for compost obtained from coniferous wood).

It has been found that the addition of calcium chloride and calcium sulphate to the mixture does not affect the period of adaptation of the micelles and the time of sporophores formation.

Tyrosinase isolated and purified from mushroom bodies showed high phenol degradation activity – 71% at the seventy-second hour.

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