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Decolorization of high concentrations of synthetic dyes by the white rot fungus *Bjerkandera adusta* strain CCBAS 232

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Abstract

Among thirty different basidiomycetes screened, *Bjerkandera adusta* strain CCBAS 232, *Phanerochaete chrysosporium* strain CCBAS 571 and *Pleurotus ostreatus* strain CCBAS 473 showed the best decolorization properties. *B. adusta* strain CCBAS 232 was able to decolorize a number of chemically different synthetic dyes (Orange G, Amaranth, Remazol Brilliant Blue R, Cu-phthalocyanine and Poly R-478) at relatively high concentrations of 2–4 g L^{-1} both on solid and in liquid medium. This strain also grew easily and produced biomass under these conditions. Unlike *P. chrysosporium* and *P. ostreatus*, *B. adusta* was able to efficiently decolorize all tested dyes. These properties and also a good ligninolytic enzyme production predetermine *B. adusta* strain CCBAS 232 for biotechnological applications.

Keywords: *Bjerkandera adusta*; Decolorization; Ligninolytic enzymes; *Phanerochaete chrysosporium*; *Pleurotus ostreatus*; Synthetic dyes

1. Introduction

In recent years, synthetic dyes are extensively used in a number of industries, such as textile dyeing or paper printing. Synthetic dyes represent a large group of chemically different compounds, which are classified by their chromophore as azo, anthraquinone, triphenylmethane, heterocyclic or phthalocyanine dyes. Due to their low biodegradability, they cause serious environmental pollution. Most dyes are very stable to light, temperature and microbial attack, making them recalcitrant compounds. Many of them are also toxic or carcinogenic. Conventional wastewater treatment systems are often inefficient and existing physical and chemical technologies are expensive, time-consuming and often methodologically demanding. One of the possible alternatives for treatment of textile effluents is the use of ligninolytic fungi, which produce various isoforms of extracellular oxidases including laccase, Mn peroxidase (MnP) and lignin peroxidase (Lip). These enzymes together with H_2O_2-producing oxidases and secondary metabolites are involved in the degradation of lignin and xenobiotic compounds including synthetic dyes.

One of the strains with high dye-degrading abilities is *Bjerkandera adusta* [1,2]. Although this fungus belongs to the well-known and frequently studied species [3–5], in the field of biodegradation more attention is paid to other species, especially *Phanerochaete chrysosporium* or *Pleurotus ostreatus* [6–10]. Nevertheless, in all these fungi the dyes were tested in the concentrations less than 1 g L^{-1}.

In the current work we studied thirty basidiomycete species for their ability to decolorize two chemically different synthetic dyes: Orange G and Remazol Brilliant Blue R (RBBR). Among them, *B. adusta* together with *P. chrysosporium* and *P. ostreatus* showed the best decolorization properties. The aim of our present work was to characterize the decolorization capacity of the white rot fungus *B. adusta* strain CCBAS 232 with stress on higher dye concentrations than are usually used and to compare its abilities with those of *P. chrysosporium* strain CCBAS 571 and *P. ostreatus* strain CCBAS 473. We used five synthetic dyes belonging to different chemical groups to reveal the differences in decolorization efficiency. As all these species easily

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decolorize a number of different synthetic dyes in lower concentrations [4,7,11–13], we searched for the limits of their decolorization capacity. The presented work brings new knowledge about synthetic dye decolorization abilities of *B. adusta*, which seems to be promising for further biotechnological exploitation.

2. Materials and methods

2.1. Organisms

All the strains (listed in Table 1) were obtained from the CCBAS collection (Institute of Microbiology AS CR, Prague, Czech Republic). The strains were maintained by serial transfers and kept on wort agar slants at 4 °C.

2.2. Chemicals

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 3-methyl-2-benzothiazolinone hydrazone (MBTH), 3-dimethylaminobenzoic acid (DMAB), Remazol Brilliant Blue R (RBBR) and Poly R-478 were purchased from Sigma; Orange G, Amaranth and Cu-phthalocyanine from Fluka. All chemicals were of analytical grade.

2.3. Culture conditions

Static cultivation was carried out in 250-mL Erlenmeyer flasks with 50 mL of N-limited (0.2 g L^{-1} of ammonium tartrate) Kirk medium [14] at 25 °C. The medium was supplemented with the respective dye at a final concentration of 2, 3 and 4 g L^{-1}. The flasks were inoculated with five wort agar plugs (10 mm diameter) cut from an actively growing part of a colony on a Petri dish. Decolorization and enzyme production were followed over a 28-day period and measured on the 7th, 14th, 21st and 28th days of cultivation.

Cultivation on solid media was carried out at 25 °C in Petri dishes (90 mm diameter) containing N-limited Kirk medium with the respective dye at a final concentration of 2, 3 and 4 g L^{-1}. The dishes (four parallels) were inoculated with mycelial plugs (3 mm diameter) cut from actively growing mycelia.

2.4. Decolorization assays

Decolorization of the liquid medium was measured in the filtrates (four parallel flasks) after removing the mycelia and

Table 1
Decolorization of synthetic dyes Orange G and RBBR by different basidiomycetes

Strain	Orange G		RBBR	
	Growth (mm)[a]	Decolorization (mm)[b]	Growth (mm)[a]	Decolorization (mm)[b]
Agaricus bisporus CCBAS 305	26	0	25	32
Agaricus xanthodermus CCBAS 225	12	0	10	33
Agrocybe cylindracea CCBAS 312	75	0	65	65
Armillaria melea CCBAS 330	17	0	17	17
Bjerkandera adusta CCBAS 232	90	90	90	90
Ceriporia metamorphosa CCBAS 269	90	0	90	90
Clitocybe gallinacea CCBAS 342	5	0	8	30
Collybia confluens CCBAS 354	12	0	7	0
Coprimus atramentarius CCBAS 356	28	0	17	17
Ganoderma lipsiense CCBAS 746	50	0	27	0
Hapalopillus rutilans CCBAS 544	11	0	25	25
Hericium clathroides CCBAS 548	35	35	25	23
Inonotus andersonii CCBAS 557	80	0	73	0
Inonotus obliquus CCBAS 559	55	0	45	45
Kuehneromyces mutabilis CCBAS 383	40	40	35	40
Laccaria proxima CCBAS 146	40	40	30	40
Lentinus edodes CCBAS 389	56	30	40	33
Lepista saeva CCBAS 401	34	0	18	25
Phanerochaete chrysosporium CCBAS 571	90	90	90	90
Phellinus pomaceus CCBAS 265	36	28	17	10
Phellinus punctatus CCBAS 262	37	37	32	34
Pholiota adiposa CCBAS 683	50	35	40	27
Pholiota lenta CCBAS 780	60	60	35	35
Pleurotus ostreatus CCBAS 473	90	90	90	90
Polyporus brumalis CCBAS 589	30	0	30	30
Polyporus ciliatus CCBAS 592	90	90	70	70
Pycnoporus sanquineus CCBAS 596	90	75	90	90
Schizophyllum commune CCBAS 752	85	0	68	0
Stropharia semiglobata CCBAS 504	50	50	33	35
Tyromyces lacteus CCBAS 616	90	45	90	90

[a] Radial growth rate measured as a diameter of mycelial colony on the 14th day of cultivation on Kirk N-limited medium containing 0.2 g L^{-1} of the respective dye.
[b] Decolorization measured as a diameter of decolorized zone on a Petri dish on the 14th day of cultivation on Kirk N-limited medium containing 0.2 g L^{-1} of the respective dye.

monitored spectrophotometrically at the maximum visible wavelength of absorbance (478 nm for Orange G, 525 nm for Amaranth). Systems without fungus served as an abiotic control. Decolorization activity was tested also on solid media, where the radial growth and the zone of color change on the agar plates were measured daily. All measurements were repeated three times.

2.5. Ligninolytic enzyme assays

Enzyme activity was measured in filtrates from four parallel flasks detained after mycelia removal. Activities of extracellular laccase (EC 1.10.3.2) and manganese peroxidase (EC 1.11.1.13, MnP) were determined spectrophotometrically by monitoring the absorbance increase at 425 nm (laccase) or 590 nm (MnP) in the reaction mixture. Laccase activity was assayed according to Bourbonnais and Paice [15] by monitoring the oxidation of ABTS. Determination of MnP activity using MBTH and DMAB was based on the method of Ngo and Lenhoff [16] modified according to Daniel et al. [17]. MBTH and DMAB were oxidatively coupled by the action of the enzyme in the presence of added H_2O_2 and Mn^{2+} ions to give a purple indamine dye product. The values were corrected for the activities in the test samples (nonspecific peroxidase activity) without manganese where manganese sulfate was substituted by ethylenediaminetetraacetate (EDTA) to chelate Mn^{2+} ions present in the extract. All measurements were repeated three times. One unit of enzyme activity (U) was defined as an amount catalyzing the production of 1 μmol of green or purple dye per mL per min. Activity of lignin peroxidase (LiP) was determined spectrophotometrically by monitoring the oxidation of veratryl alcohol in the presence of H_2O_2 according to Tien and Kirk [18].

2.6. Determination of growth and biomass production

Radial growth was estimated by measuring the diameter of the colonies grown on the solid agar medium on Petri dishes. Biomass production in liquid media was evaluated by determining the dry mass of mycelia. Mycelia were harvested from the cultivation flasks, washed with distilled water, dried at 105 °C for 24 h and weighed.

3. Results and discussion

3.1. Screening of basidiomycete strains for decolorization of Orange G and RBBR

Thirty different basidiomycete strains were tested for decolorization and radial growth rate on agar plates containing 0.2 g L^{-1} of Orange G or RBBR (Table 1). All strains were able to grow on solid media in the presence of the dyes, but only 15 (50%) strains decolorized both the dyes tested. Four (13%) strains decolorize neither Orange G nor RBBR while eleven (37%) strains decolorized only RBBR. We did not find any strain decolorizing only Orange G. The strains that did not decolorize the dyes within 14 days (see Table 1) showed the same results also after 20 days of cultivation. Out of the tested strains, we found the highest decolorization capacity in *B. adusta*, *P. chrysosporium* and *P. ostreatus*. A similar test system using two structurally different dyes (azo and anthraquinone dyes) has been successfully used also by

Table 2
Decolorization of synthetic dyes by *B. adjusta*, *P. chrysosporium* and *P. ostreatus* growing on agar plates

Dye	Concentration (g L^{-1})	Growth (mm, %)[a]			Decolorization (mm, day)[b]		
		B. adjusta	*P. chrysosporium*	*P. ostreatus*	*B. adjusta*	*P. chrysosporium*	*P. ostreatus*
Orange G (azo dye)	2	75 (88)	90 (100)	65 (81)	90 (6)	90 (6)	0
	3	75 (88)	90 (100)	62 (78)	60 (6)	90 (6)	0
	4	72 (85)	90 (100)	57 (71)	50 (6)	90 (6)	0
Amaranth (azo dye)	2	85 (100)	90 (100)	80 (100)	75 (6)	90 (3)	25 (8)
	3	85 (100)	90 (100)	75 (94)	74 (6)	80 (6)	0 (10)
	4	85 (100)	90 (100)	62 (78)	50 (6)	70 (6)	0 (12)
RBBR (anthraquinone dye)	2	24 (29)	90 (100)	12 (15)	11 (6)	0	0
	3	17 (20)	15 (17)	10 (13)	0 (26)	0	0
	4	15 (18)	12 (13)	8 (10)	0 (26)	0	0
Cu-phthalocyanine (phthalocyanine dye)	2	85 (100)	90 (100)	78 (98)	65 (6)	80 (6)	0
	3	80 (94)	90 (100)	75 (94)	50 (6)	0	0
	4	75 (88)	90 (100)	50 (63)	15 (8)	0	0
Poly R-478 (polyaromatic dye)	2	85 (100)	90 (100)	62 (78)	75 (3)	90 (3)	0
	3	85 (100)	90 (100)	78 (98)	74 (6)	80 (3)	0
	4	85 (100)	90 (100)	75 (94)	50 (6)	50 (3)	0

[a] The first number represents the diameter of the mycelial colony in mm (measured on the 8th day of cultivation), the number in parentheses shows the colony diameter in percentage of the control (control = colony diameter of fungus (measured on the 8th day of cultivation) growing on the Kirk N-limited agar medium without dyes; it was 85 mm for *B. adusta*, 90 mm for *P. chrysosporium*, 80 mm for *P. ostreatus*).

[b] The first number shows the diameter of the decolorized zone in mm (measured on the 8th day of cultivation), the number in parentheses indicates the day of cultivation on which the decolorization started.

other authors [5,19]. Our results revealed that the majority of the strains decolorized RBBR (anthraquinone dye) more easily than Orange G (azo dye). These findings are in correspondence with literature data [5,20].

3.2. Decolorization capacity of *B. adusta* in comparison with *P. chrysosporium* and *P. ostreatus* during cultivation on solid medium

B. adusta, which belongs to the strains with the best decolorization properties among our strains tested (see Table 1), was chosen for detailed studies. Although this strain is well studied [2,4,21], the majority of the authors tested the synthetic dyes only at concentrations of 0.1–0.5 g L^{-1} [2,3,5]. As we did not find any information reporting on decolorization of higher concentrations of synthetic dyes in either *B. adusta* or in other fungi, we performed an agar-plate experiment with different synthetic dyes at concentrations of 2–4 g L^{-1}. For comparison we made the same experiment also with *P. chrysosporium* and *P. ostreatus*, which showed similar decolorization abilities like *B. adusta* in our screening (see Table 1) and their biodegradation properties and decolorization capacity are well studied.

The results summarized in Table 2 revealed a very high decolorization ability of *B. adusta*. The majority of the tested dyes (except for RBBR) were efficiently decolorized on solid media even at a concentration of 4 g L^{-1} (see Table 2). RBBR was partly decolorized only at a concentration of 2 g L^{-1}. Moreira et al. [21] thoroughly studied decolorization of RBBR by *Bjerkandera* sp. and concluded that the concentration of RBBR strongly influenced decolorization activity of the strain. RBBR decolorization rate increased with the

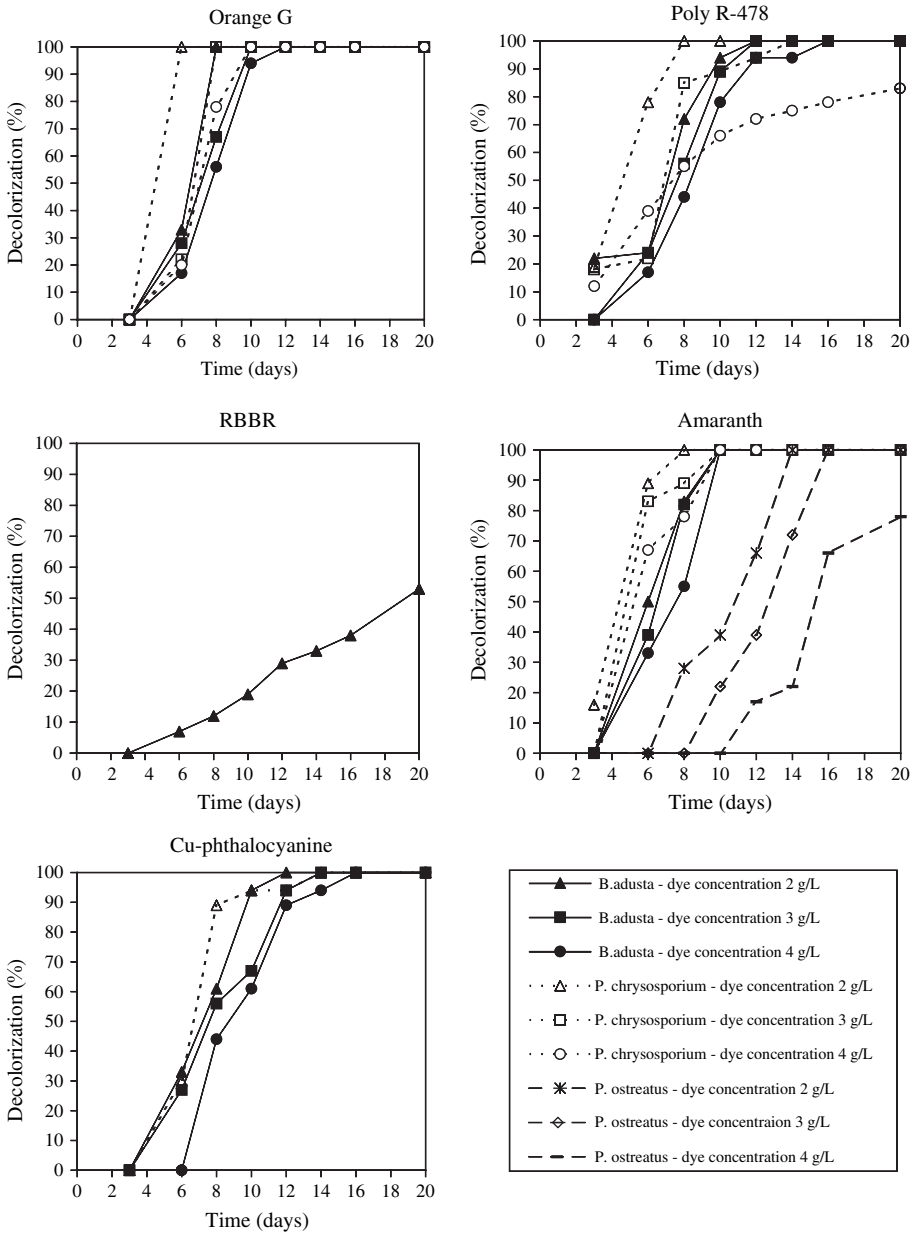

Fig. 1. Decolorization of synthetic dyes by *B. adusta*, *P. chrysosporium* and *P. ostreatus* on Petri dish (in percentage of Petri dish diameter, which was 90 mm).

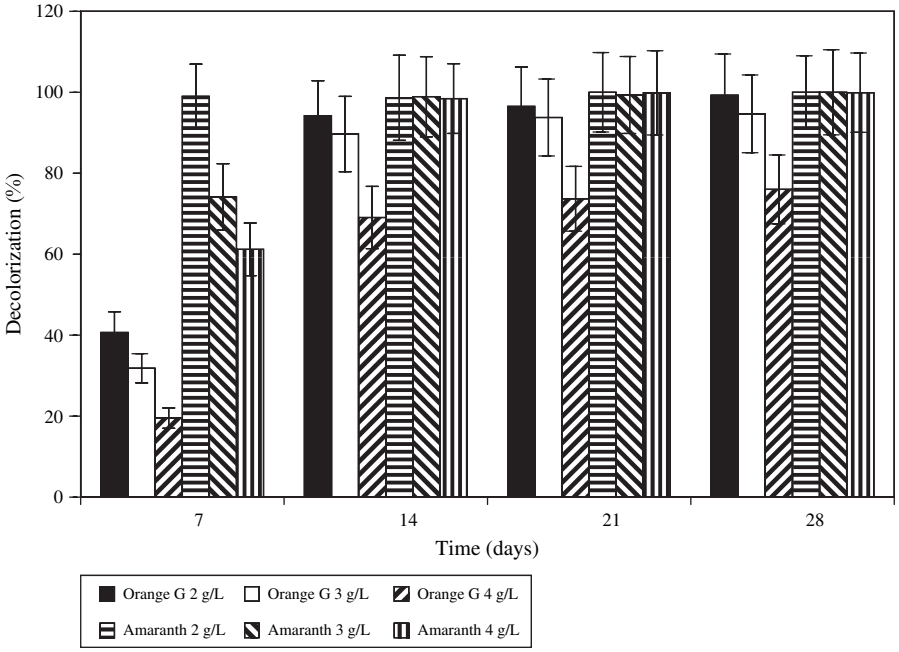

Fig. 2. Decolorization of Orange G and Amaranth by *B. adusta* in liquid culture (in percentage of initial amount of each dye).

increase of RBBR concentration only up to a certain level. Further increase in RBBR concentration inhibited decolorization. Out of all studied dyes, Amaranth and Orange G were decolorized most rapidly in all the cases. The decolorization started usually on the 6th day of cultivation. Easy decolorization of these two azo dyes is somewhat surprising, because azo dyes are, due to their chemical structure, not readily degraded and are considered to be resistant to decolorization [22,23].

Although the presence of synthetic dyes often reduced the mycelial growth rate of fungi, we found a substantial inhibition of growth only in the case of RBBR. No or only a slight growth reduction was found in case of Orange G, Amaranth, Cu-phthalocyanine or Poly R-478 even at a concentration of 4 g L^{-1} (see Table 2).

The results described above (see Table 2) indicate that only *B. adusta* was able to decolorize all tested dyes (in case of RBBR with a limiting concentration 2 g L^{-1}). In case of Orange G, Amaranth and Poly R-478 decolorization capacity of *P. chrysosporium* was comparable with *B. adusta*. However, *P. chrysosporium* did not decolorize RBBR at all and Cu-phthalocyanine only up to the concentration of 2 g L^{-1}. *P. ostreatus* exhibited very limited decolorization abilities; Amaranth was the only dye decolorized by this strain (see Table 2). The decolorization time courses for individual dyes show that increasing concentrations of the dyes correlate with their slower decolorization in all the cases (Fig. 1). Nevertheless, the majority of the dyes were completely decolorized within 10–12 days. *B. adusta* rapidly decolorized all the dyes, except for RBBR, the decolorization rate of which was slower. Amaranth was the most rapidly decolorized dye and it was also the only dye decolorized by all three tested strains; *P. chrysosporium* exhibited the highest and *P. ostreatus* the lowest decolorization rate. Also in case of Orange G and at a lower concentration of Poly R-478 (2 g L^{-1}) we found the highest decolorization rate in *P. chrysosporium*, while a higher concentration of Poly R-478 (4 g L^{-1}) was more rapidly decolorized by *B. adusta.*

3.3. Decolorization of synthetic dyes by B. adusta in liquid culture

Two most rapidly decolorized dyes on solid medium (Orange G and Amaranth) were used also for decolorization experiments in liquid culture. Results showed that also under these conditions both dyes in concentrations of 2–4 g L^{-1} were efficiently decolorized by *B. adusta* (Fig. 2). The early step in azo dyes' decolorization – the breaking of the azo bond – and also further degradation involving aromatic cleavage depend on the identity, number and position of functional groups in the aromatic region and the resulting interaction with the azo bonds [24–26]. Orange G has an aromatic ring and two sulfonic groups less than Amaranth [12].

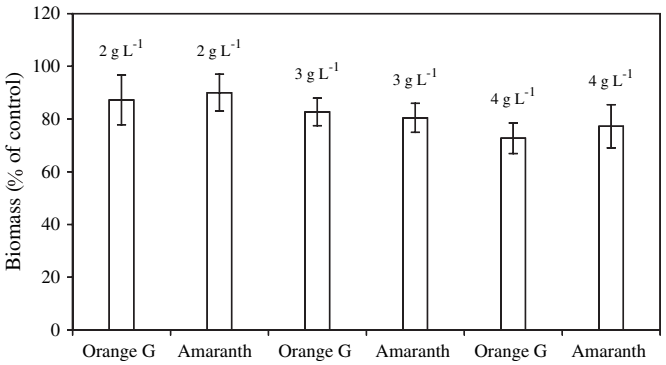

Fig. 3. Biomass production of *B. adusta* in liquid medium with dyes (data are expressed in percentage of control, where the control was the amount of biomass of *B. adusta* growing on medium without dye; control = 4.4 g L^{-1}).

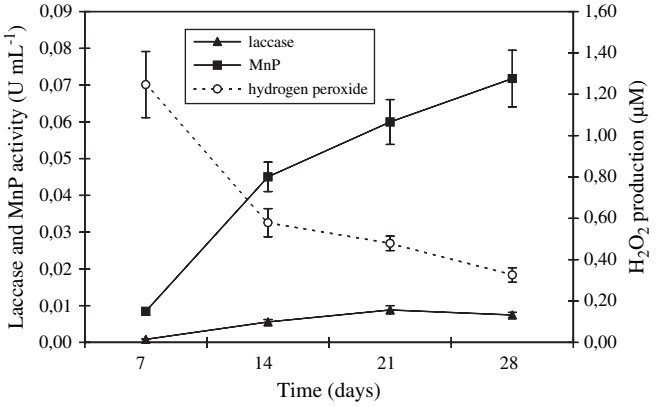

Fig. 4. Ligninolytic enzyme and H_2O_2 production in *B. adusta*.

Nevertheless, at the beginning of cultivation Amaranth was decolorized more rapidly than Orange G. Within 14 days of cultivation both dyes were almost completely decolorized, except for the highest concentration (4 g L^{-1}) of Orange G. In this case the decolorization rate was slightly lower and in the end of the cultivation (at day 28) only 76% of the dye was removed. From our finding we can conclude that the overall complexity alone is not an indicator of the difficulty of decolorization of a particular dye and even small structural differences can affect the decolorization process [24,25].

Increasing concentration of dyes brings about only a slight decrease in growth and biomass production (Fig. 3). At the end of the cultivation (after 28 days) the culture containing Orange G at a concentration of 4 g L^{-1} had the lowest biomass production (73%) in comparison with the control culture growing in the medium without dyes. It correlates with lower decolorization of this sample. The role of biomass production in fungal decolorization of dyes becomes significant if the process requires biomass-associated factors such as H_2O_2 or if certain degradative enzymes are bound to the mycelium [26].

Several papers emphasize the role of MnP and LiP in synthetic dye decolorization by *B. adusta* [2,21]. Under our experimental conditions (static cultivation), we found production of laccase and MnP, but no detectable activity of LiP in our strain of *B. adusta* (Fig. 4). As the activity of MnP was several times higher than that of laccase, we suppose that MnP is more important, although it is highly probable that both these enzymes took a certain role in decolorization of synthetic dyes by *B. adusta*. Nevertheless, this process is obviously more complicated and is influenced by many other factors such as radicals, different mediators, hydrogen peroxide, etc. [27–29].

4. Conclusion

Our results show that the white rot fungus *B. adusta* is capable of decolorizing a number of chemically different synthetic dyes, even at relatively high concentrations. The strain has a substantially higher decolorization capacity than *P. ostreatus* and comparable decolorization ability with *P. chrysosporium*. *B. adusta* easily grew on media containing high concentrations of dyes and showed a good biomass production even under these conditions. All these properties of *B. adusta* together with its ligninolytic enzyme production indicate that this strain could be interesting for biotechnological exploitation.

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