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ORIGINAL PAPER

Comparison of efficiency for monoazo dye removal by different species of white-rot fungi

V. Pecková[1] · B. Legerská[1] · D. Chmelová[1] · M. Horník[2] · M. Ondrejovič[1]

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Abstract

The aim of this study was to determine the potential of white-rot fungi, namely *Pycnoporus cinnabarinus*, *Pleurotus ostreatus* and *Trametes hirsuta*, for the mono azo dye Allura Red AC (AR) removal from aqueous solutions. AR belongs to the hardly degradable xenobiotic associated with a neurotoxic effect on humans and animals. Our results suggested that degradation processes driven by the activity of laccases were not involved in the process of AR removal and the predominant mechanism of dye elimination was biosorption. The surface of fungal biomass was analyzed by Fourier transform infrared spectroscopy (FTIR) and Langmuir and Freundlich models of absorption isotherms were applied to describe the biosorption isotherms. Langmuir model fitted the equilibrium data better than Freundlich isotherm according to the corrected Akaike Information Criterion (AIC_c). From Langmuir model, dead biomass of *P. ostreatus* modified by heat was the most suitable biosorbent with the maximum sorption capacity of 118.3 ± 9.9 mg/g dried biomass. Obtained results suggest that biomass of white-rot fungi can be used as a suitable and low-cost biosorbent for the removal of azo dyes from contaminated waters.

Keywords Removal · Biosorption · Azo dye · Biomass · White-rot fungi · Adsorption isotherms

Introduction

The extensive application of synthetic dyes in a wide range of industry results in their release into the environment (Yang et al. 2016). It has been assumed that 10–25% of synthetic dyes are not used in the dyeing process and 2–20% is directly released into the wastewater streams (Zahria and Suteu 2012). Dyeing industry, especially the textile industry, belongs to the major producer of wastewater containing synthetic dyes (Sen et al. 2016). Azo dyes represent approx. 70% of all synthetic dyes used in dyeing industry and can be characterized by one or more chromophores (–N=N–) linked to benzene or naphthalene rings with the side chains of –OH, $–SO_3H$ or other functional groups (Solís et al. 2012; Thiam et al. 2015a). The presence of azo dyes in wastewaters poses a potential risk to living organisms (Sharma et al. 2007; Thiam et al. 2015b) due to their toxic, carcinogenic or mutagenic effects (Saratale et al. 2011; Nath et al. 2015; Gopinathan et al. 2015). Therefore, the removal of the dyes has become a major research topic (Legerská et al. 2016, 2018).

The monoazo dye Allura Red AC (AR) belongs to hardly degradable xenobiotic associated with a neurotoxic effect on humans and animals (McCann et al. 2007; Noorafshan et al. 2018). The effective degradation of AR was obtained by solar photoelectron-Fenton physico-chemical method (Thiam et al. 2015a, b) although, the presence of persistent toxic oxalic acid as degradation product was observed (Thiam et al. 2015a). Moreover, traditional physical, physico-chemical and chemical techniques are less effective methods for dye elimination due to chemical stability of dyes and their resistance to degradation or the materials and equipment improving process efficiency are too expensive for their commercial purposes (Zhao et al. 2006; Vanhulle et al. 2008; Nguyen and Juang 2013). Therefore, the environmentally acceptable biological methods of dye elimination

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✉ D. Chmelová
daniela.chmelova@ucm.sk

[1] Department of Biotechnology, Faculty of Natural Sciences, University of Ss. Cyril and Methodius in Trnava, Nám. J. Herdu 2, SK-917 01 Trnava, Slovakia

[2] Department of Ecochemistry and Radioecology, Faculty of Natural Sciences, University of Ss. Cyril and Methodius in Trnava, Nám. J. Herdu 2, SK-917 01 Trnava, Slovakia

appear to be more promising than conventionally used methods (Forgacs et al. 2004).

Biological methods involve the use of microorganisms and/or their metabolites in dye removal. The uptake of dye by microbial biomass occurs in three possible ways: (1) biosorption of dye onto the surface of microbial biomass; (2) biodegradation of dye molecules by ligninolytic enzymes such as laccases and peroxidases; and (3) bioaccumulation of dye in living cells by transport membrane systems (Kaushik and Malik 2009; Yang et al. 2016; Sen et al. 2016). White-rot fungi belong to perspective microorganisms for this purpose (Yang et al. 2016; Legerská et al. 2018). The use of fungal biomass for dye elimination has some advantages, namely a relatively inexpensive process, low operating costs and less production of sludge (Forgacs et al. 2004; Sen et al. 2016). For white-rot fungi, dye degradation by ligninolytic enzymes and biosorption are recently widely studied (Aksu et al. 2010; Ramírez-Montoya et al. 2015; Legerská et al. 2016; Huang et al. 2016). However, biodegradation of azo dyes by fungal ligninolytic enzymes requires the enzymes with high redox potential (+730 to +790 mV vs. normal hydrogen electrode; NHE) (Yang et al. 2015) but the toxicity of degradation products should be evaluated (Thiam et al. 2015a; Legerská et al. 2018). *Pycnoporus cinnabarinus*, *Pleurotus ostreatus* and *Trametes hirsuta* were used as the model fungal producers of high redox potential laccases with 750, 740 and 780 mV vs. NHE, respectively (Li et al. 1999; Garzillo et al. 2001; Shleev et al. 2005). In biosorption, the dye molecules are not degraded or transformed, but dyes are adsorbed onto the surface of biomass/biosorbent. As a result, the dyes are removed from the aqueous solution, but the problem with high amounts of dyes trapped in biomass/biosorbent still remains. The traditionally used sorbents such as activated carbon have several disadvantages such as the relatively high cost of pretreatment, difficult regeneration and the reduction of sorption efficiency after the regeneration (Srivastava et al. 2007). Therefore, other suitable biosorbents for synthetic dyes removal from wastewaters based on microbial or plant biomass are intensively investigated (Lei et al. 2014; Jain and Gogate 2018; Crominski da Silva et al. 2019). White-rot fungi producing the significant biomass yields seem to be useful for this purpose (Marcharchand and Ting 2017; Kumar et al. 2018; Przystaś et al. 2018). *P. cinnabarinus*, *P. ostreatus* and *T. hirsuta* are well-known fast-growing fungi (Freitag and Morell 1992; Levasseur et al. 2014; Sabantina et al. 2019).

The aim of this work was to evaluate the potential of fungal biomass (*P. cinnabarinus* DSM 3022, *P. ostreatus* DSM 1833 and *T. hirsuta* DSM 3491) for the removal of the hard-to-degrade monoazo dye AR from aqueous solutions. The contribution of ligninolytic enzymes, namely laccases, to the AR elimination was estimated. For the evaluation of biosorption efficiency, Langmuir and Freundlich adsorption isotherms were used and surface properties of fungal biomass were characterized by Fourier transform infrared spectroscopy (FTIR).

Materials and methods

Chemicals and synthetic dye

All chemicals used in the experiments were of analytical grades. The azo dye Allura Red AC (AR; C.I. 16,035; MW 496.4 g/mol) was purchased from Sigma-Aldrich (Germany) (Fig. 1). The wavelength (λ_{max}) with the absorption maximum of this azo dye was determined by spectrophotometer UV-1600 PC (VWR, Germany).

Microorganisms and culture conditions

The white-rot fungi, namely *Pycnoporus cinnabarinus* DSM 3022, *Pleurotus ostreatus* DSM 1833 and *Trametes hirsuta* DSM 3491 were purchased from German Collection of Microorganisms and Cell Cultures GmbH (Germany). The cultures were maintained on malt agar supplemented with agar (1%, *w/v*) at 4 °C. For the fungal biomass collection, the strains were re-inoculated and incubated at 30 °C on malt agar. The fungal biomass of 7-day-old culture was collected (6×1 cm^2 squares), added to sterile distilled water (20 mL) and stirred at 150 RPM for 30 min. Aliquot amount of the fungal biomass (2 mL) was used to inoculate the culture medium (20 mL) containing glucose (10 g/L), casein hydrolysate (5 g/L), $MgSO_4$. 7 H_2O (0.5 g/L), NaCl (0.1 g/L), $CuSO_4$. 5 H_2O (0.1 mg/L), $FeSO_4$. 7 H_2O (0.2 mg/L), $MnSO_4$. 4 H_2O (0.02 mg/L) and $ZnCl_2$ (0.15 mg/L) in 100 mmol/L phosphate buffer (pH 5.0) at 30 °C and shaking of 140 RPM for 14 days under submerged conditions. This composition of culture medium and cultivation conditions were used in all experiments.

Enzyme decolorization

The potential of laccases produced by white-rot fungi to eliminate AR was verified in culture media with the addition of AR in the concentration range of 25 − 250 mg/L.

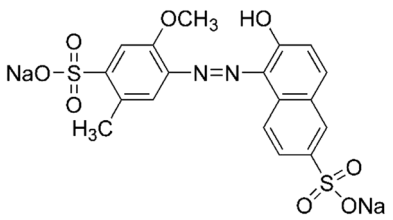

Fig. 1 The structure of the azo dye Allura Red AC

Laccase activity was monitored during the whole cultivation and after 14 days, the amount of removed dye was calculated from the difference between the initial dye concentration in the abiotic control without fungal biomass and the dye concentration in the culture medium after 14 days of cultivation. The azo dye AR concentration was calculated from the calibration curve measured at a wavelength of 493 nm (spectrophotometer UV-1600 PC; VWR, Germany).

In individual experiments, the decolorization of AR was also studied using the crude laccase extract. This was obtained after the removal of biomass from the culture medium without the addition of AR. The supernatant was centrifuged at 4000 RPM for 10 min. Decolorization potential of prepared crude laccase extract with the activity of 1.0 U/mL was tested in 100 mmol/L phosphate buffer (pH 5.0) containing AR (initial concentration of 100 mg/L) during 5 days at 20 °C and compared to the control. The control was prepared by boiling the culture medium supernatant at 100 °C for 10 min to denature the proteins.

Sorption decolorization

The sorption potential of both living and dead fungal biomass (*P. cinnabarinus*, *P. ostreatus* or *T. hirsuta*) for AR elimination from aqueous solutions was verified. Fungal biomass for the sorption experiments was obtained after 14-day cultivation under above-mentioned conditions. After this time, the culture media were decanted, and fungal biomass was repeatedly washed with the phosphate buffer (100 mmol/L; pH 5.0). In the case of living fungal biomass, the experiments were carried out in the flasks with 20 mL solution containing AR in the concentration range of 15–340 mg/L dissolved in 100 mmol/L phosphate buffer (pH 5.0) with the addition of 0.01% (*w/v*) sodium azide (for suppression of laccase activity).

The study of AR biosorption by dead biomass was performed under identical conditions as in the case of living biomass, but the biomass was heat-pretreated. Produced biomass in phosphate buffer (100 mmol/L; pH 5.0; 20 mL) was boiled for 10 min at 100 °C. Subsequently, the flasks with dead biomass were cooled, the phosphate buffer was decanted, and AR dissolved in 100 mmol/L phosphate buffer (pH 5.0) was added in the concentration range of 20–355 mg/L. The sorption efficiency was evaluated as decolorization of AR (in %; Eq. 1) and in terms of adsorbed amount of azo dye (in mg/g; Eq. 2).

$$\text{Decolorization} = 100 \cdot \left(\frac{c_0 - c_t}{c_0} \right) \tag{1}$$

where c_0 is the initial dye concentration (mg/L) and c_t is the dye concentration (mg/L) in the selected time interval. The amount of adsorbed azo dye was calculated according to Eq. 2.

$$Q_t = \frac{(c_i - c_e) \cdot V}{N} \tag{2}$$

where Q_t is the amount of the azo dye AR adsorbed per gram of living/dead biomass of the selected white-rot fungus (*P. cinnabarinus*, *P. ostreatus* or *T. hirsuta*) (mg/g; d.w.); c_i is the initial concentration of dye (mg/L), c_e is the residual dye concentration at equilibrium (mg/L); N is the mass of dry biomass (g); and V is the solution volume (L).

At the time of reaching the equilibrium, the sorption process was terminated, and obtained data were evaluated by adsorption isotherms according to the Langmuir and Freundlich. Specific sorption (mg/g; d.w.) was evaluated using adsorption isotherms by the Langmuir (Eq. 3) and Freundlich (Eq. 4) models (Freundlich 1906; Langmuir 1918).

$$Q_{\text{eq}} = \frac{b \times Q_{\max} \times C_{\text{eq}}}{1 + b \times C_{\text{eq}}} \tag{3}$$

$$Q_{\text{eq}} = K \times C_{\text{eq}}^{\left(\frac{1}{n}\right)} \tag{4}$$

where Q_{eq} represents the specific dye sorption on the biomass of the white-rot fungus (mg/g d.w.); b is Langmuir equilibrium constant describing the affinity between sorbent (biomass) and sorbate (dye) (L/mg); $Q_{\max}$ is the maximum sorption capacity of fungal biomass (mg/g, d.w.); C_{eq} represents the residual concentration of dye in a solution at equilibrium (mg/L), K represents Freundlich equilibrium constant describing the sorption capacity of fungal biomass (L/g); and n is dimensionless Freundlich constant expressing the sorption intensity.

FTIR analysis

After 14 days of cultivation in the culture medium, the biomass was heat-pretreated and after this, the biomass was repeatedly washed with deionized water and dried at 50 °C to constant weight. The dried samples were homogenized, mixed with KBr and pressed into the pellets by a manual hydraulic press Specac 15 for analysis. The spectra were acquired in triplicates in the range of wavelength of 400–4000 cm^{-1} using a Tensor 27 FTIR spectrometer (Bruker, Billerica, MA, USA).

Laccase activity

The activity of laccases was determined as follows: 150 µL of a 1 mmol/L solution of ABTS (2-2′-azinobis (3-ethylbenzothiazoline-6-sulfonate)) in 0.2 mol/L phosphate buffer (pH 5.0) and 50 µL of the sample were mixed. The

mixture was then incubated for 10 min to determine the change in absorbance caused by oxidation of ABTS by laccases in the plate reader at 450 nm. Enzyme activity was expressed in U/mL. 1 U is defined as the enzyme activity that catalyses the conversion of 1 µmol ABTS in 1 min.

Statistical methods

Individual experiments were performed in triplicate. The experimental data were processed using OriginPro 2016 (OriginLab Corporation, Northampton, MA, USA). The corrected Akaike information criterion (AIC_c) (Akaike 1974) and Akaike weight (λ_i) by Akpa and Unuabonah (2011) were used to verify better sorption isotherm model.

Results and discussion

Combined processes of AR elimination

The removal of AR from aqueous solutions by white-rot fungi takes place by biosorption, enzymatic degradation by ligninolytic enzymes such as peroxidases and laccases or the combination of both mechanisms (Ramírez-Montoya et al. 2015; Legerská et al. 2016; Huang et al. 2016). The production of peroxidases by *P. cinnabarinus*, *P. ostreatus* and *T. hirsuta*, namely lignin and manganese peroxidases, was not confirmed in our previous works (Hazuchová et al. 2017; Pecková and Chmelová 2019). For this reason, laccase activity and removed dye concentration were determined after 14 days of submerged cultivation of selected white-rot fungi (Fig. 2). The utilization of glucose used as a carbon source in the culture media and the maximal biomass production

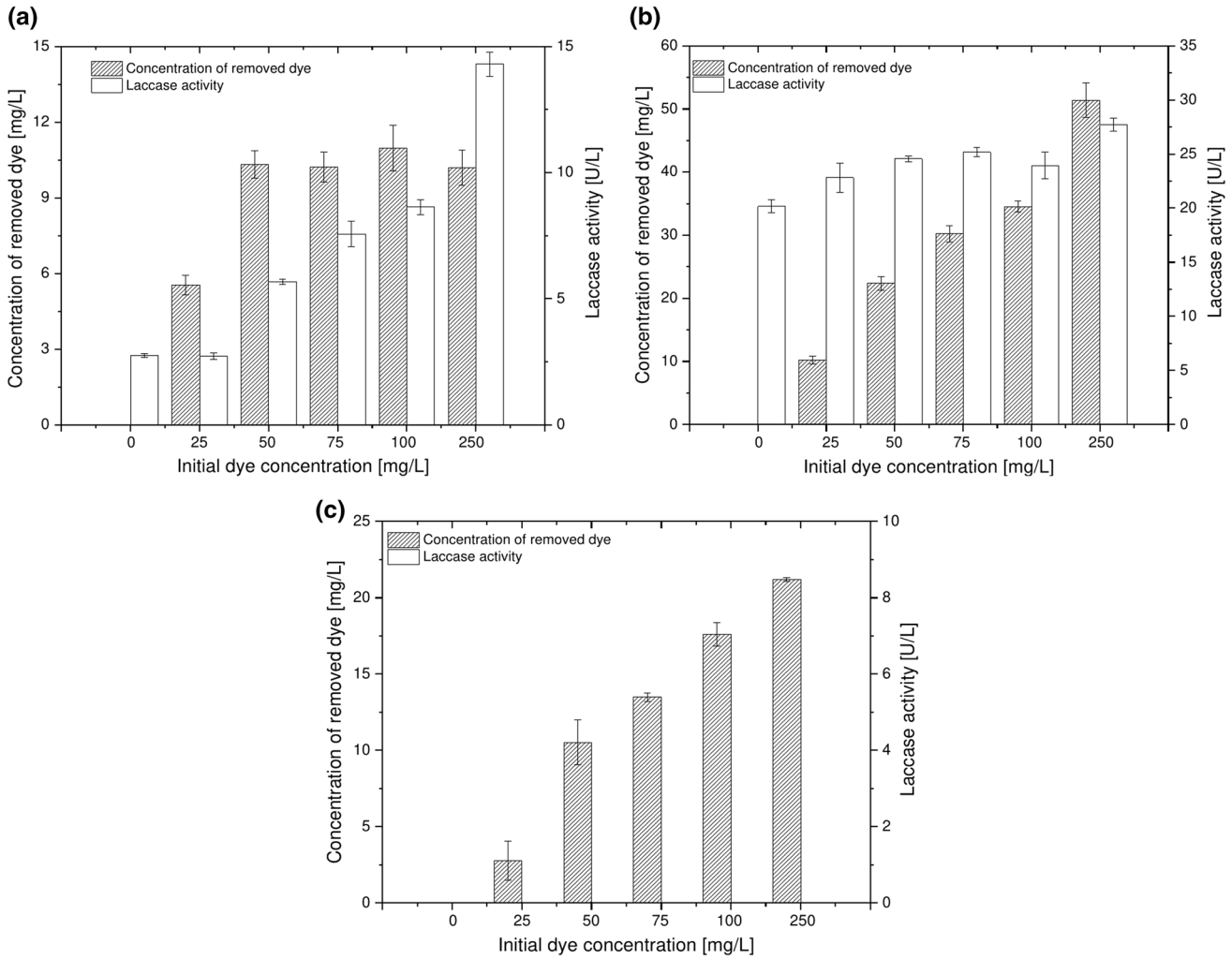

Fig. 2 Concentration of removed AR from culture media containing living biomass of *P. cinnabarinus* (**a**), *P. ostreatus* (**b**) or *T. hirsuta* (**c**) and laccase activity determined after 14 days of cultivation under submerged conditions (30 °C, initial pH 5.0, 140 RPM)

were the parameters determining the cultivation time (data not shown).

During the cultivation, all studied white-rot fungi produced laccases, except for *T. hirsuta* (Fig. 2). Moiseenko et al. (2018) observed that *T. hirsuta* produces several extracellular laccases depending on the conditions of production and the composition of the culture medium. It was found that the depletion of carbon source in culture media inducted the production of laccase in the case of *P. cinnabarinus*. On the contrary to this, the laccase activity was determined during the whole time of *P. ostreatus* cultivation (data not shown). The amount of removed AR in culture media increased in order: *P. cinnabarinus* < *T. hirsuta* < *P. ostreatus.* The lack of *T. hirsuta* laccase production suggests that the decrease of AR concentration was caused by the sorption processes of AR binding onto the surface of this fungal biomass (Fig. 2c). The observed data of both laccase activity and removed dye concentration in *P. cinnabarinus* culture media allow us to conclude that the predominant mechanism of AR elimination was biosorption. Laccase activity increases with increasing the initial dye concentrations in the range of 50–250 mg/L, but the concentrations of removed AR are comparable to each other (10.2–11.0 mg/g d.w.) (Fig. 2a). Although, the sorption typically represents a rapid process with reaching the higher specific sorption capacities with increasing the concentration of synthetic dye (Aksu et al. 2008, 2010), the sorption process can be stopped when all binding sites located on the biomass surface are fully occupied (Zeroual et al. 2006; Aksu et al. 2010; Bouras et al. 2017). It can be assumed, for *T. hirsuta* (Fig. 2c) results, all binding sites located onto the biomass surface were occupied at higher dye concentrations. The role of *P. ostreatus* laccase in the process of AR elimination along with sorption processes must be considered. It was found that there is a linear dependence between the concentration of removed dye and laccase activity produced by *P. ostreatus* ($R^2 = 0.8$). The highest values of laccase activity were measured at the highest AR concentration (250 mg/L; 27.7 ± 0.6 U/L) and the concentration of removed AR increased with increasing the *P. ostreatus* laccase activity (Fig. 2b).

Enzyme catalyzed elimination of AR

Therefore, the crude enzyme extracts of laccase-producing *P. cinnabarinus* and *P. ostreatus* were tested for AR decolorization during the 5 days of the exposure (Fig. 3).

The results of AR decolorization by crude laccase extract of *P. cinnabarinus* and *P. ostreatus* did not confirm the contribution of laccases to dye elimination (Fig. 3). It is known that laccases produced by *P. cinnabarinus* and *P. ostreatus* belong to laccases with high-redox potential (Garzillo et al. 2001; Shleev et al. 2005). On the contrary, high-redox potential laccases were not able to degrade AR (Fig. 3), but the dye structure itself also plays an important role in the removal of the dye by laccases. AR is one of the azonaphthalene dyes containing a single azo bond (Fig. 1). Electron-acceptor groups such as $–SO_3H$ are more resistant to enzyme degradation compared to electron-donor groups such as –OH (Hsueh et al. 2009; Solís et al. 2012). Dye biodegradation by enzymes can be increased using appropriate redox mediators (Sen et al. 2016). Some white-rot fungi can produce low-molecular weight compounds (e.g., vanillin, acetosyringone, phenolic acids) that can act as potential redox mediators (Polak and Wilkolazka 2012). These can be produced under negative environmental conditions including the presence of an azo dye in a medium. Although, the used crude laccase extract (Fig. 3) was prepared from a culture medium without the addition of the dye and therefore, the production of low-molecular weight compounds may not have started at all. From the measured results (Fig. 2b), it can be assumed

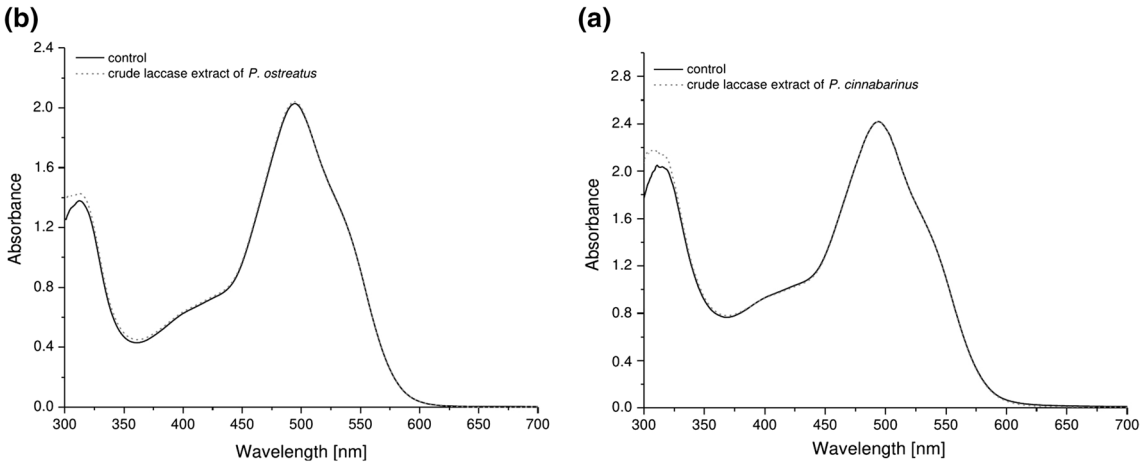

Fig. 3 The effect of crude laccase extracts of *P. cinnabarinus* (**a**) and *P. ostreatus* (**b**) on AR decolorization (100 mg/L) after 5 days of incubation at 20 °C and pH 5.0

that laccase mediator system of *P. ostreatus* can be partially involved in the AR removal process. However, sorption seems to be the dominant process of AR elimination for all fungi used (Figs. 2, 3). In the next step, we focused on testing the sorption ability of fungal biomass to remove AR, but the production of laccases by living biomass was suppressed by the addition of sodium azide.

Biosorption of AR

Dead and live fungal biomass

Adsorption isotherms provide the information about the properties of biosorbents from the point of view of their sorption efficiency (Hasan et al. 2012; Akar et al. 2013). Several factors affect the effectiveness of biosorption, but the method of pretreatment of microbial biosorbent has a significant effect on the biosorption rate (Taha et al. 2014; Huang et al. 2016). In general, the sorption of xenobiotics by dead biomass is more effective than by living biomass. Therefore, we compared the biosorption efficiency of living biomass and heat-treated dead biomass of studied white-rot fungi to remove AR from aqueous solutions (Fig. 4).

It was found that the percentage of AR decolorization was increased with increasing the dye concentration, although, the highest AR concentrations had the negative effect on this parameter (Fig. 4). A similar trend was observed in studies of Aksu et al. (2010) and Chakraborty et al. (2013). The best potential for AR removal was demonstrated by living biomass of the white-rot fungus *P. ostreatus*, which reached the maximum decolorization efficiency of 26.9% (Fig. 4c). However, the highest decolorization efficiency, as well as the highest sorption potential, was observed in the case of all heat-treated biomass studied. In the comparison of applied white-rot fungi, the biomass *T. hirsuta* showed the ability to remove more than 53% of AR from the initial amount in the medium (Fig. 4f). Considering these facts, it can be concluded that heat treatment of fungal biomass probably improved the sorption properties of biomass as a sorbent. This phenomenon could be explained by a change in the porosity of prepared biosorbent increasing the specific surface for AR sorption. As the biosorbent, heat-treated biomass has an advantage over living cells, since the dead biomass, in general, cannot be affected by the toxic effects of the dye (Arica and Bayramoglu 2007). Our results suggest that heat-treated biomass of studied white-rot fungi shows better potential to remove synthetic dyes than living biomass (Fig. 4).

Modeling the biosorption equilibrium

The surface properties and biosorbent efficiency can be assessed by adsorption isotherms (Hasan et al. 2012; Akar et al. 2013). Langmuir or Freundlich adsorption isotherm models (Freundlich 1906; Langmuir 1918) are often used for the description of the dye removal by fungal biomass (Patel and Suresh 2008; Yang et al. 2011; Taha et al. 2014; Lei et al. 2014; Huang et al. 2016). The most appropriate parameters of Langmuir and Freundlich models for the characterization of biosorbent sorption capacity are Q_{max} and Freundlich constant K which represent the maximum sorption capacity and sorption capacity of the given biosorbent, respectively. Predicted values of individual parameters, as well as the correlation coefficients R^2, obtained from the description of the data using nonlinear regression method are presented in Table 1.

As can be seen from Table 1, the coefficients of determination (R^2) for both living and dead biomass were higher for the Langmuir than Freundlich model of adsorption isotherm, suggesting that AR sorption onto fungal biomass can be described by monolayer sorption according to the model theory. These conclusions are also supported by the corrected Akaike information criterion (AIC_c) that belongs to an appropriate statistical tool for the search of the best model for one dataset. Langmuir model has the minimum values of AIC_c for all tested variables and the value of λ_i, which shows of how many times it is more likely that the chosen model is the correct model, suggests that the Langmuir model is a better model describing the AR sorption onto the surface of fungal biomass (Table 1).

From obtained results, the heat-treated biomass showed a higher maximum sorption capacity for all fungal biomass than for living biomass. This fact is also confirmed by higher affinity of the dead fungal biomass to the AR sorbate expressed by Langmuir constant of b. The Freundlich model assumes that dye sorption occurs on a heterogeneous surface associated with interactions between sorbed molecules (Freundlich 1906). The values of the coefficients of determination as well as the constant K suggest that this model does not fit the sorption process well (Table 1). The value of $1/n$ is related to the strength of adsorption, and if this value is less than one, we are talking about normal adsorption and if it is higher than one, it is cooperative adsorption. Normal adsorption was observed in the cases of living and dead fungal biomass and cooperative adsorption was not observed in our experiments (Table 1).

Lei et al. (2014) described that the Langmuir isotherm model fitted the biosorption process of the Neutral Red dye by *P. ostreatus* well with the maximum sorption capacity of 61.7 mg/g. On the base of our results, it can be concluded that the dead biomass of *T. hirsuta* and *P. ostreatus* represent the interesting sorbents due to the amount of sorbed/removal of dye (Table 1). Table 2 summarizes the maximum sorption capacities Q_{max} of different types of sorbents for synthetic dyes removal predicted from the Langmuir model of adsorption isotherm. From the comparison of mentioned values, it

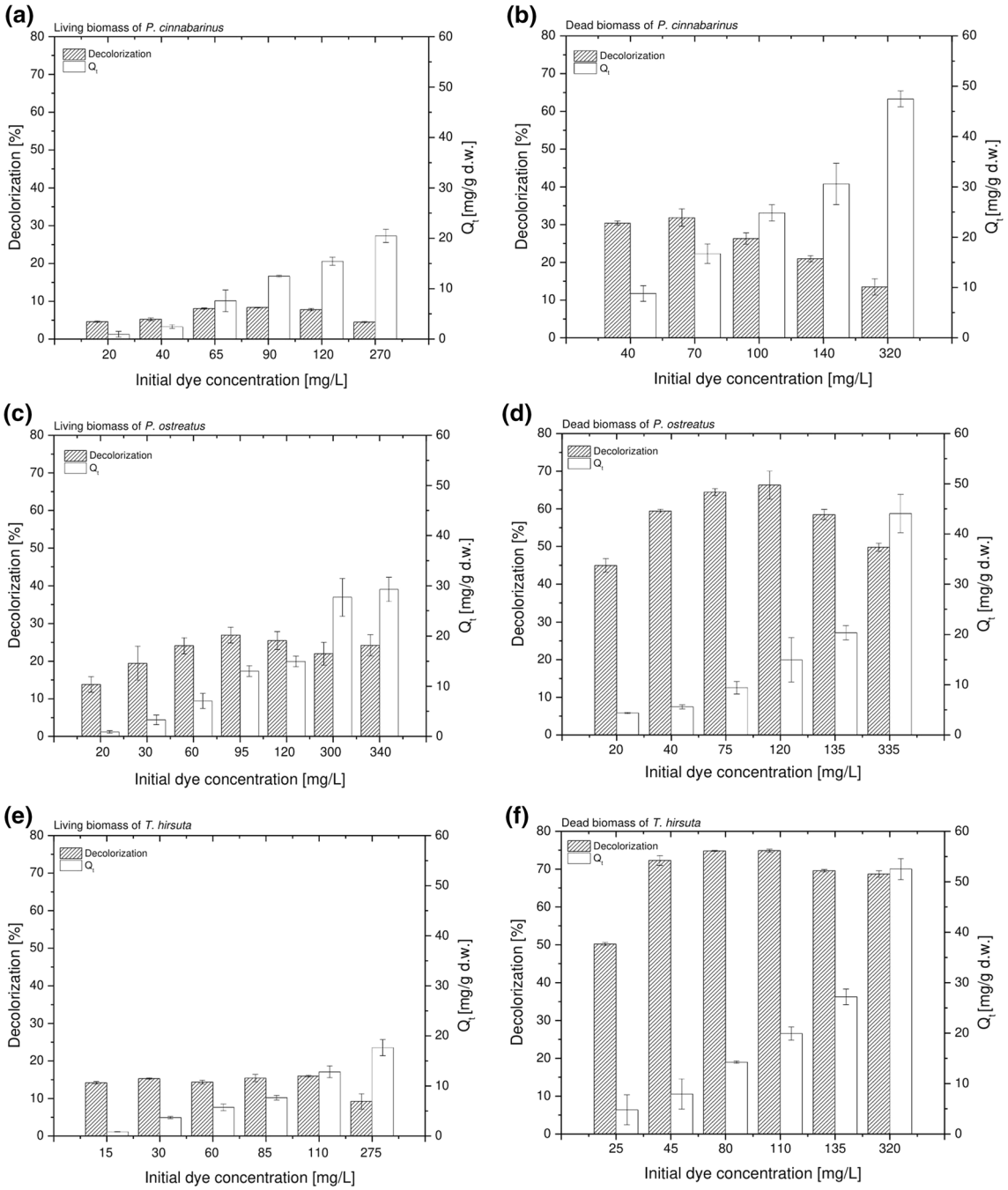

Fig. 4 Efficiency of decolorization and specific capacity for removal of AR from aqueous solutions by living and dead biomass of selected white-rot fungi. (Q_t) of AR in the concentration range 15–340 mg/L was carried out for 1 h at 20 °C in phosphate buffer (100 mmol/L; pH 5.0). Legend: Q_t—the amount of the azo dye AR adsorbed per gram of living/dead biomass of the selected white-rot fungus

is evident that the studied fungal biomass modified by heat-treatment method shows comparable and, in many cases, higher sorption capacities for synthetic dyes removal. Heat-treated biomass of *P. ostreatus* showed 1.9-times higher sorption capacity than dried *P. ostreatus* at 40 °C (Table 2). It can be assumed that exposure of *P. ostreatus* biomass to high temperature for a relatively short time improves the sorption properties of the fungal biomass. Fungal biomass appears to be a better sorbent compared to plant biosorbents, except of dried leaves of *Ficus racemosa* pre-treated by NaOH. However, the sorbent makes the sorption process expensive due to the consumption of chemicals as well as water needed to neutralize the biosorbent. The traditionally used sorbents such as graphene oxide and zeolite showed

Table 1 Biosorption parameters for the removal of AR by living and dead biomass of selected white-rot fungi obtained by nonlinear regression using Langmuir and Freundlich adsorption isotherms and the comparison of isotherm models

White-rot fungus	Biomass	Langmuir model					Freundlich model				
		Parameters					Parameters				
		Q_{max} (mg/g d.w.)	b (L/mg)	R^2	AIC$_c$	λ_i	n	K (L/g)	R^2	AIC$_c$	λ_i
Pycnoporus cinnabarinus	Living biomass	41.3 ± 15.8	0.004 ± 0.003	0.91	26.56	0.77	1.42 ± 0.33	0.44 ± 0.36	0.87	28.98	0.23
	Dead biomass	77.0 ± 6.1	0.006 ± 0.001	0.99	31.30	0.97	1.70 ± 0.20	1.79 ± 0.61	0.97	38.43	0.03
Pleurotus ostreatus	Living biomass	70.3 ± 14.6	0.003 ± 0.001	0.99	16.90	0.87	1.29 ± 0.13	0.41 ± 0.16	0.98	20.63	0.13
	Dead biomass	118.3 ± 9.9	0.004 ± 0.001	0.99	10.91	0.98	1.26 ± 0.06	0.77 ± 0.13	0.99	18.46	0.02
Trametes hirsuta	Living biomass	32.6 ± 8.6	0.005 ± 0.002	0.95	21.10	0.80	1.49 ± 0.27	0.46 ± 0.27	0.92	23.92	0.20
	Dead biomass	107.5 ± 6.1	0.008 ± 0.001	0.99	25.44	0.98	1.67 ± 0.14	2.41 ± 0.67	0.98	32.74	0.03

Table 2 The comparison of maximum sorption capacity for the elimination of different types of synthetic dyes using various adsorbents

Sorbent	Modification	Synthetic dye	Q_{max} (mg/g)	References
P. cinnabarinus	Heat-treated	Allura Red AC	77.0 ± 6.1	This work
T. hirsuta	Heat-treated	Allura Red AC	107.5 ± 6.1	This work
P. ostreatus	Heat-treated	Allura Red AC	118.3 ± 9.9	This work
P. ostreatus	Dried	Neutral Red	61.7	Lei et al. (2014)
Seedcake from *Salvia hispanica*	Dried	Reactive Yellow B2R	76.3	Crominski da Silva and Teixeira de Abreu Pietrobelli (2019)
Dried leaves of *Prunus dulcis*	NaOH treatment	Acid Green 25	50.8	Jain and Gogate (2018)
Dried leaves of *Ficus racemosa*	NaOH treatment	Acid Violet 17	119.1	Jain and Gogate (2017)
Dried leaves of *Ficus racemosa*	H_2SO_4 treatment	Acid Violet 17	61.4	Jain and Gogate (2017)
Graphene oxide*		Basic Red 12	43.5	Robati et al. (2016)
Graphene oxide*		Methyl Orange	83.3	Robati et al. (2016)
Zeolite	Modified with HMDA	Reactive Red 239	29.7	Alver and Metin (2012)
Zeolite	Modified with HMDA	Reactive Blue 250	58.5	Alver and Metin (2012)

Legend: *HMDA* hexymethylenediamine. *Single layer graphene oxide

the lower sorption capacity of azo dyes than fungal biomass used in this study (Table 2).

The main advantages of biosorption are high selectivity, cost-effective process without the formation of sludge, simple dye desorption and biosorbent utilization in several cycles (Yang and Feng 2010; Xin et al. 2012; Rybczyńska-Tkaczyk and Korniłłowicz-Kowalska 2016; Chen et al. 2019). The application of dead fungal biomass is more preferred for hardly degradable dyes such as AR than living biomass. Dead fungal biomass is not affected by the toxicity of wastewater and it needs to supply no nutrients to ensure growth and metabolism (Arica and Bayramoglu 2007). However, depending on the dye structure, it is also possible to use a combined dye removal process with enzyme catalysed reactions and sorption (Pecková et al. 2018). The patent literature describes the possibilities of using lignolytic enzymes, especially laccases, in the removal of lignin or its modifications, biocatalysis, production of various substances usable in pharmacy, medicine, and the food industry, production of biosensors and bioremediation and biodetoxification. An overview of patent literature on this topic was compiled by Zerva et al. (2019). Some patents also focus on the use of biomass of white-rot fungi in the removal of textile dyes from the environment (US20020151038; WO2006059348A1). The white-rot fungi are non-pathogens to humans and can be produced by simple fermentation process or obtained as the waste from industrial fermentation processes. Moreover, the reusability of fungal sorbents is a key factor to make the economical treatment process. Si et al. (2014) found slight loss in adsorption capacity of *T. pubescens* dead biomass after three cycles. Sorption efficiency of immobilized *T. viride* biomass decreased about 4.3% after five adsorption–desorption cycles of methylene blue (Saeed et al. 2009). It appears that fungal biomass is

a suitable environmentally acceptable alternative for dye biosorption.

FTIR analysis

Dye sorption, and biosorption in general, is associated with the interaction between cell walls of fungi and their components and sorbates -molecules of synthetic dyes. Fungal cell walls contain chitin and chitosan molecules, amino acids, lipids, fatty acids, and various surfactant groups (carboxyl and carbonyl groups, phosphate groups) that are involved in binding of synthetic dye molecules (Kaushik and Malik 2009; Yang et al. 2016). The structure of the dye itself (molecular weight, presence of functional groups and polarity) can affect the sorption efficiency of the dye. The presence of functional groups present on the mentioned cell walls components and their quantitative distribution determine the extent of interactions between the dye molecule and cell wall. FTIR analysis was carried out on all tested heat-treated fungal samples (Fig. 5).

In the region of valence vibration of X–H (4000–2500 cm^{-1}), a strong, wide band in the region of 3500–3300 cm^{-1} representing the –OH group was found. The functional group was identified in FTIR spectra of all tested fungi (Fig. 5). The stretching vibration at 3500–3200 cm^{-1} represents amino groups (Lei et al. 2014). C–H stretch in the region at 2900–2800 cm^{-1} was also observed. The valence vibrations of the C–H aliphatic compounds are usually assigned to two bands. The corresponding vibration for the CH_2 group was found at ~2930 and ~2860 cm^{-1}. FTIR spectrum of *P. ostreatus* (Fig. 5) shows a weak band at 2407 cm^{-1} in the triple-bond region of the spectrum (2500–2000 cm^{-1}) and the band was not found in FTIR spectra of *P. cinnabarinus* and *T. hirsuta*.

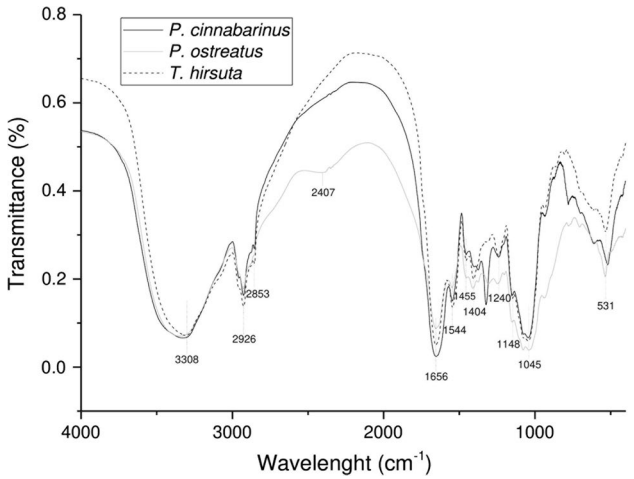

Fig. 5 FTIR spectra for heat-treated dead biomass of *P. cinnabarinus* (**a**), *P. ostreatus* (**b**) and *T. hirsuta* (**c**)

The region between 2000 and 1500 cm^{-1} is characteristic for double-bond vibrations (C=C, C=O, C=N and N=O). The band at ~1650 cm^{-1} is relevant to C=C and C=O bonds. The valence vibrations of COO^- ion are characterized by two strong bands on the spectra at 1610–1550 cm^{-1} and 1420–1300 cm^{-1}. The fingerprint region (1500–600 cm^{-1}) shows the bands at 1044, 1041 and 1045 cm^{-1} for the biomass of *P. cinnabarinus*, *P. ostreatus* and *T. hirsuta*, respectively. These bands can be assigned to C–O or C–C stretching vibrations. The bands in the region of 1180–820 cm^{-1} are associated with carbohydrates of cell walls, such as glucan and chitin (Nie et al. 2007; Szeghalmi et al. 2007). Stretching of P=O and P-OH represents absorption peaks at 1150 and 1079 cm^{-1}. The peaks at 520–536 cm^{-1} can correspond to aromatic components. The deformation vibration (612–520 cm^{-1}) represents C=N=C group. Also, in the region of 1654–1041 cm^{-1} of all IR spectra were identified the differences in the spectra of the white-rot fungi tested (Fig. 5). IR spectrum of *P. ostreatus* did not confirm the presence of peaks identified in the case of *P. cinnabarinus* and *T. hirsuta* biomass. Moreover, the region between 1700 and 2800 cm^{-1} for heat-treated *P. ostreatus* biomass shows the different profile than these regions of *P. cinnabarinus* and *T. hirsuta*. The above-mentioned bands in fungal biomass FTIR profiles suggest the presence of s –NH, –OH, C=O, P=O, P–OH, and C=N=C groups probably participated in the sorption process. FTIR analysis indicates the possible involvement of various functional groups. Chen et al. (2019) suggested that hydroxyl, carbonyl, amino, phosphoryl and nitro groups are mainly involved in dye biosorption. The changes in band intensity and wave values suggested that carboxyl, hydroxyl and amino groups are involved to azo dye uptake by fungal biomass (Ghariani et al. 2019).

Conclusion

The elimination of synthetic dyes used in various industrial applications by environmentally acceptable methods is one of the recent research topics. Sorption represents rapid processes compared to enzymatic reactions, when the concentration equilibrium between sorbent and sorbed dye in the solution can be reached within several minutes or hours. Obtained results showed that the pretreatment of white-rot fungi biomass by heat caused the changes in the surface properties and structure of such biosorbent which led to increased sorption capacity. In this context, it was found that dead biomass of *P. ostreatus* and *T. hirsuta* had the comparable or higher sorption capacities for synthetic dye removal than recently tested biosorbents derived from the plant biomass or inorganic sorbents, such as zeolites. Heat-treated fungal biomass appears to be a suitable biosorbent for those synthetic dyes recalcitrant to enzymatic degradation.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest for the authors.

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