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# Removal of a textile dye (RBBR) from the water environment by fungi isolated from lignocellulosic composts

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**Keywords:** water environment, textile dye, *Trichoderma* sp, peroxidase activity, Remazol Brilliant Blue R.

**Abstract:** A representative group of hydrophilic fungi from the genus *Trichoderma* isolated from lignocellulose composts with varying degrees of maturity was analyzed for their ability to biodegrade a harmful anthraquinone dye, i.e. Remazol Brilliant Blue R (RBBR). In RBBR-containing post-culture liquids, there were determined the degree of RBBR decolorization, horseradish peroxidase-like, superoxide dismutase-like, and xylanase activities, and the concentrations of low-molecular phenolic compounds.

The study results demonstrated that *Trichoderma asperellum*, *T. harzianum*, and *T. lixii* strains isolated from compost containing larger amounts of easily available lignocellulose fractions, i.e. grasses, exhibit higher RBBR decolorization efficiency ranging from 0.3 to 62% than *T. citrinoviride* strains isolated from compost II, which contained greater quantities of hardly degradable lignocellulose. The decolorization of remazol blue R by the investigated *Trichoderma* strains intensified significantly with the increase in peroxidase activity and it was correlated with a decline in the content of low-molecular phenolic compounds. The dynamics of changes in the horseradish peroxidase-like, superoxide dismutase, and xylanase activities in the aqueous post-culture liquids of the investigated fungal strains depended largely on the duration of the culture.

Given their ability to adapt to water environments, e.g. wastewater, and to decolorize and detoxify the RBBR anthraquinone dye, *Trichoderma* fungi can be used for bioremediation of such environments.

## Introduction

Inherent elements in the textile industry are the burdensome and harmful by-products, which are still an unsolved problem due to the lack of safe methods for their disposal and management thereof (Shedbalkar et al. 2008).

Besides processing raw materials into fibers, fabrics, and knitwear, the textile industry produces large quantities of colored waste by-products due to the large amounts of water used during the production cycle (Singh et al. 2015). The growing demand for water and its decreasing supply necessitates treatment and reuse of industrial wastewater (Megha et al. 2015).

Such wastewater may contain from 2% of basic fabric-unbound dyes and up to 50% of reactive dyes, which may contribute to serious pollution of ground- and surface waters, although, as shown by Jin et al. (2007), 280,000 tons of dyes are removed annually from wastewater of the textile industry (Singh et al. 2015). Azo and anthraquinone dyes such as remazol blue (RBBR) are extremely harmful reactive dyes (Rodrigez-Couto et al. 2011). The remazol blue textile dye destroys living organisms upon entering waters (Singh et al. 2015). As shown by Bhatt et al. (2000) and Mester and Tien (2000), textile dyes structurally resemble polycyclic aromatic hydrocarbons (PAHs) and lignin subunits. The current physicochemical methods for the removal of these contaminants are not environmentally friendly, as they contribute to the formation of colorless toxic secondary products, e.g. benzidine (Singh et al. 2015). Although they allow for effective degradation of organic pollutants, electrochemical technologies consume more energy (Zou et al. 2019). Alternative environmentally friendly approaches to decolorization of dye-contaminated wastewater include biological methods. At present, many research centers in the Poland (Rybczyńska and Korniłłowicz-Kowalska 2015; Jasińska et al. 2012) and abroad (López et al. 2006, 2007; Sumandono et al. 2015) search for efficient microbial strains that can be used in effective decolorization and detoxification of post-industrial wastewater. Several potential candidates have been identified among fungi, bacteria, yeast, and algae with a high ability to decolorize many anthraquinone dyes (Singh et al. 2015). The involvement of white rot fungi of the genera *Phanerochaete*, *Bjerkandera*, and *Trametes* in the processes of modification of anthraquinone dyes, with particular emphasis on RBBR, has been reported in recent years by e.g. Sumandono et al. (2015) and Korniłłowicz-Kowalska and Rybczyńska

(2012). The ability to biodegrade RBBR is also tested in micromycetes, as reported by Jasińska et al. (2012) and Noman et al. (2019), in *Staphylococcus* sp. bacteria (Velayutham et al. 2018), and in bacterial and/or fungal consortia (Khudhair et al. 2015; Lade et al. 2016).

Microorganisms that grow well in the water environment are especially valuable organisms playing an important role in the biodetoxification of colored industrial wastewater. They include representatives of the *Trichoderma* genus, which were shown by Grabińska-Łoniewska (2004) as hydrophilic well--growing and well-sporulating fungi in water environments including wastewater. Among them, fungi isolated from natural environments that are rich in lignocellulosic organic matter seem to be very promising in this regard. There is a relationship between the ligninolytic abilities of fungi and the abilities to biodegrade other aromatic compounds, including colored ones, as reported by Ulmer et al. (1984). López et al. (2006) and Chamuris et al. (2000) suggest that micromycetes isolated from composts can be potential wood lignocellulose degraders. In turn, Ryazanova et al. (2015) have demonstrated that, besides their ability to decompose cellulose, *Trichoderma* fungi have the ability to degrade lignin. The involvement of *Trichoderma* in decolorization and biodegradation of post-industrial lignin has been reported by Rybczyńska-Tkaczyk and Korniłłowicz-Kowalska (2017).

Given some reports on the involvement of micromycetes in the biological modification of colored contaminants in wastewater and the potential ligninolytic abilities of *Trichoderma* fungi, the aim of the study was to assess the possibility of modification of structurally lignin-related Remazol Brilliant Blue R (RBBR) by *Trichoderma* strains isolated from composts with varying maturity. This is a new approach to look for microbial strains that can be useful for elimination of post-industrial dyes present in wastewater before introduction thereof into waters.

## Materials and methods

### *Composts as a source of potentially ligninolytic fungi*

*Trichoderma* fungi were isolated from two lignocellulose composts with a varied maturation degree composted for 10, 20, and 30 weeks.

Compost I (PGSF) contained 42.86% of pine bark, 34.28% of grass, 20.00% of sawdust, and 2.86% of broiler chicken feathers, C/N=25.

Compost II (PSSF) contained 25.54% of pine bark, 10.63% of wheat straw, 51.07% of sawdust, and 12.76% of broiler chicken feathers, C/N=25. A detailed characterization of the composting process is presented in a study conducted by Bohacz (2017).

Fungi with potential ability to biodegrade lignocellulose, including fungi of the genus *Trichoderma*, were isolated on Petri dishes where 5 g of each type of compost was sprinkled with powdered industrial lignin (9 dishes for each type of the compost). After a 10-week incubation at 26°C, mycelia were transferred onto agar medium containing 0.02% RBBR and 0.25% glucose as described by Korniłłowicz-Kowalska et al. (2008). Strains that exhibited RBBR decolorization abilities on Petri dishes were checked for their ability to decolorize RBBR and release ligninolytic enzymes as well as low-molecular compounds in liquid cultures of these fungi.

*Trichoderma* strains were selected for the study of ligninolytic activity, as they were most abundant among all the isolated micromycetes and constituted over 30% in compost I and approx. 50% in compost II (unpublished data).

### *Identification of Trichoderma strains*

Preliminary identification of six fungal strains (designated VII, XII, XIX, VI, VIII, and XXV) was based on macro- and microscopic features, i.e. the colony morphology, the size and shape of spores, and the structure of the conidiophore. The observations were carried out using an Olympus BX-41 microscope with a CVIII4 digital camera integrated with a computer and the Cell-A program for archiving and documenting photographs. Final verification was based on systematic keys (Domsch et al. 2007).

To confirm species affiliation, the fungi were identified by means of PCR using specific primers and sequencing of PCR templates. The sequences were deposited in the GenBank under the following accession numbers: MH571704.1 *Trichoderma harzianum* Rifai, MH 602297.1 *Trichoderma lixii* (Pat.) P. Chaverri, MH 602236.1 *Trichoderma asperellum* Samuels, Lieckf & Nirenberg, MH 602423.1 *Trichoderma citrinoviride* Bissett, MH 602287.1 *Trichoderma citrinoviride* Bissett and MH 602289.1 *Trichoderma citrinoviridae* Bissett.

Strains VII, XII, and XIX were isolated from compost I (PGSF) in composting week 10, 20, and 30, respectively. Strains VI, VIII, and XXV originated from compost II (PSSF) and were isolated in composting week 10, 20, and 30, respectively.

### *Strain cultures*

Static fungal cultures were run on the modified medium described in a paper by Korniłłowicz-Kowalska et al. (2008) with the addition of 0.02% RBBR and 0.25% glucose. The media were inoculated with 1 agar disk containing 7-day mycelium. The cultures were incubated at 28°C for 32 days. The cultures of each strain were run in triplicate.

### *Analytical methods*

The biodegradation ability of the tested *Trichoderma* strains was determined in aqueous solutions obtained from cultures supplemented with 0.02% Remazol Brilliant Blue R (RBBR, $C_{22}H_{16}N_2Na_2O_{11}S_3$) as a substrate.

After 4, 8, 12, 16, 20, 24, 28, and 32 days of culture, the percentage decolorization of Remazol Brilliant Blue R, enzyme activity, and content of low-molecular phenolic compounds were determined in the post-culture liquids, which were separated from the mycelium by filtration on Millipore filters with a pore diameter of 0.22 μm, PVDF, and diam. 33 mm.

Decolorization of RBBR as a reliable indicator of detoxification of anthraquinone dyes was determined spectrophotometrically at 595 nm. The percentage of decolorization (DEC) was calculated using the formula proposed by López et al. (2006). Horseradish peroxidase-like (HRP-like) activity was determined using o-dianisidine as a substrate in acetate buffer pH = 5.5 as shown by Claiborne and Fridovich (1979). Superoxide dismutase (SOD-like) activity was determined as proposed by Marklund and Marklund (1974) and modified by Paździoch-Czochra et al. (2003) using pyrogallol as a substrate and in 0.5 M TRIS-HCl buffer, pH 8.5,

with the addition of 10mM EDTA. Xylanase activity (XYL) was determined by measuring the concentration of reducing sugars with 3.5-dinitrosalicylic acid (DNS) after degradation of xylan (Szakács Dobozi et al. 1992, Miller 1959). The protein concentration was determined by the method developed by Lowry (1951).

Phenolic compounds [hydroxyphenols (PA) and methoxyphenols (VA)] were determined spectrophotometrically at a wavelength of 400 nm and 500 nm with the methodology developed by Paździoch-Czochra et al. (2003).

The pH of the post-culture liquid was measured potentiometrically (pH-meter CP-501, Elmetron).

### *Statistical analysis*

To demonstrate the relationships between the analyzed parameters, a correlation analysis at the significance level of $p < 0.05$ was performed. Analysis of variance (ANOVA) followed by post-hoc Tukey's test for comparison of means (HSD) was used to show significant differences in the decolorization activity parameters between the strains. P values below 0.05 were considered significant.

## Results

### *Decolorization*

The results of the study demonstrated that strains VII, XII, and XIX isolated from compost containing the higher level of the easily degradable lignocellulosic component (grass) exhibited the highest efficiency in the decolorization of Remazol Brilliant Blue R. The level of decolorization ranged from 3.7% to 62.7%. *Trichoderma lixii* strain XII isolated from compost I after 20 weeks of the composting process decolorized over 60% of RBBR. *Trichoderma asperellum* strain XIX isolated after 30 weeks of composting decolorized RBBR systematically but with lower efficiency. *T. harzianum* strain VII isolated after 10 weeks of composting decolorized a maximum of 27.6% of RBBR during the first days of culture, which was followed by re-colorization and weak re-decolorization. Strains isolated from compost II (PSSF) did not exhibit the ability to decolorize RBBR, with the exception of *Trichoderma citrinoviride* strain XXV, which only transiently decolorized the dye. Medium darkening was noted in the *Trichoderma citrinoviride* strain cultures.

The Tukey HSD statistical analysis at a significance level of $\alpha=0.05$ showed that the decolorization activity of strains isolated from compost I at different experimental time points, i.e. in weeks 10 and 20, was significantly different from the activity of compost II strains isolated at the same time. The decolorization ability was significantly positively correlated with horseradish peroxidase-like activity (HRP-like) (0.329***) and negatively correlated with phenolic compounds (methoxy- and hydroxyphenols) (-0.691***; -0.599***) (Table 1).

### *Enzymatic activity*

The highest horseradish peroxidase-like (HRP-like) activity was detected in post-culture liquids of strains isolated from compost I (PGSF) (Fig. 2). High activity of this enzyme was determined in the cultures of the *Trichoderma lixii* XII and *T. harzianum* VII strains. This effect was evident at the later time points in the experiment, i.e. after 20 days of culture on the RBBR medium. The activity of this enzyme in the cultures of strains isolated from compost II was lower and differed significantly from the HRP-like activity in the strains from compost I, especially in those isolated in composting weeks 10 and 20 (Fig. 2). As demonstrated by the *r*-Pearson correlation analysis, the activity of this enzyme increased significantly with the duration of the fungal culture (0.260***) and was significantly negatively correlated with xylanase activity (-0.280***) (Table 1).

The highest xylanase activity was recorded at the beginning of the culture of all the *Trichoderma* strains, i.e. up to day 12. Afterwards, the activity of this enzyme declined significantly. The statistical analysis (Tukey's test) indicated that the xylanase activity did not differ significantly between the strains (Fig. 3). The activity of this enzyme significantly decreased with the duration of the fungal culture (-0.813***) and, as in the case of peroxidase and superoxide dismutase, was negatively correlated with phenol compounds (-0.268*** and -0.450***) (Table 1).

As shown by the data presented in Figure 4, the activity of superoxide dismutase (SOD) was generally higher in the cultures of compost I strains. The activity of superoxide dismutase in the *T. lixii* XII and *T. citrinoviride* VI, VIII, and XXV cultures reached the highest value up to culture day 16. In turn, after day 20, high activities of this enzyme were recorded in the *T. asperellum* XIX and *T. harzianum* VII cultures. Significant differences in the activity of this enzyme were recorded in the cultures of fungi isolated from compost I and II in composting week 10, i.e. between strains VII and XXV (Fig. 4).

**Table 1.** Correlation coefficients (*r*) between the analyzed traits at the significance level α=0.05, α=0.01, α=0.001; ns-not significant

| traits | time | HRP-like | SOD-like | XYL | PA | VA | pH | DEC |
|---|---|---|---|---|---|---|---|---|
| time | – | 0.260** | -0.207* | -0.813*** | 0.307*** | 0.537*** | 0.287*** | ns |
| HRP-like | | – | ns | 0,280*** | -0,190* | -0,205* | 0,415*** | 0,329*** |
| SOD-like | | ns | – | 0,206* | -0,290*** | -0,197* | ns | ns |
| XYL | | -0,280*** | 0,206* | – | -0,268** | -0,450*** | -0,267** | ns |
| PA | | -0,190* | -0,290*** | -0,268** | – | 0,797*** | -0,375*** | -0,599*** |
| VA | | -0,206* | -0,1979* | -0,450*** | 0,797*** | – | -0,222** | -0,691*** |
| pH | | 0,415*** | ns | -0,2676** | -0,375*** | -0,222** | – | 0,415*** |
| DEC | | 0,329*** | ns | ns | -0,599*** | -0,691*** | 0,415*** | – |

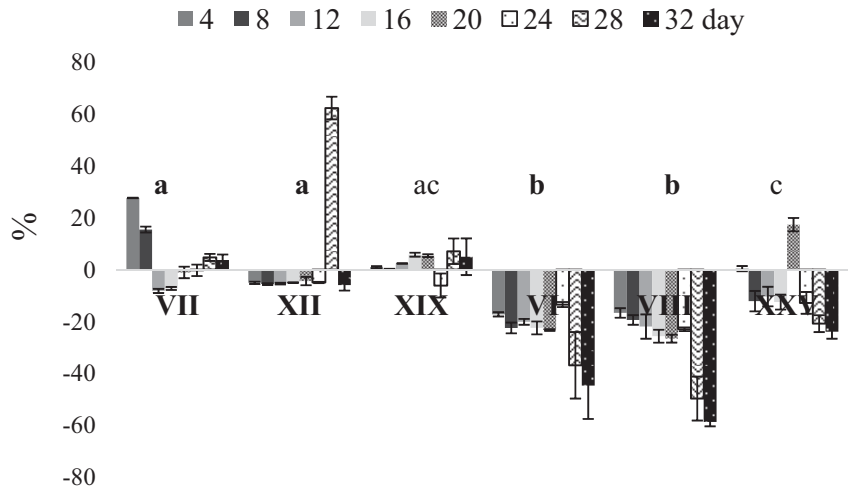


**Fig. 1.** Degree (%) of decolorization (above axis x) and re-colorization (below axis x) of 0.02% remazol blue R (RBBR) by compost-derived *Trichoderma* fungi; Explanations: Small letters (a, b, c) designate homogenous groups; means marked with the same letter (e.g., a, b, c) do not differ significantly (α=0.05). Means marked with different letters (e.g., a and b) differ significantly (α=0.05). Bars represent standard deviation (*n*=3)

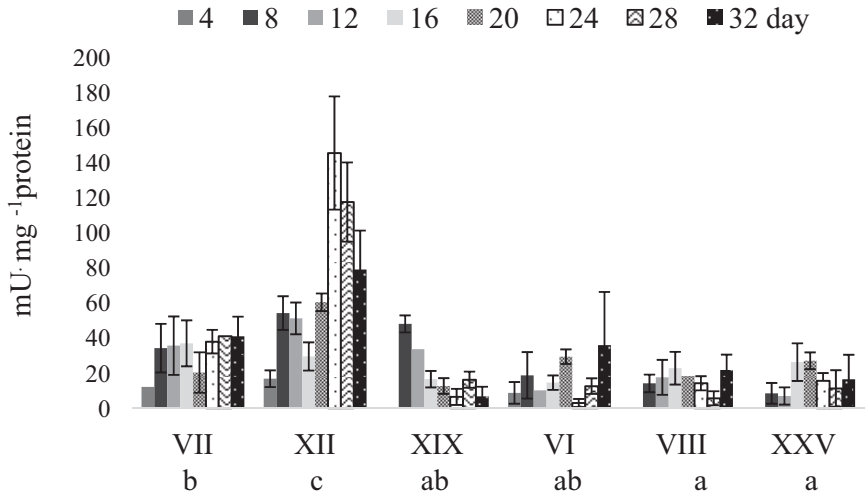


**Fig. 2.** Changes in horseradish peroxidase-like (HRP-like) activity in post-culture liquids of *Trichoderma* strains isolated from composts. Explanations as in Fig. 1

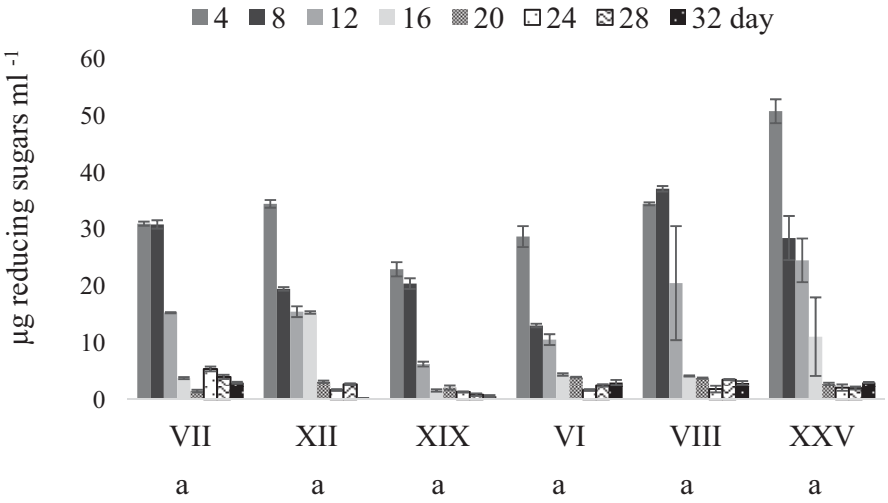


**Fig. 3.** Changes in xylanase (XYL) activity in post-culture liquids of *Trichoderma* strains isolated from composts. Explanations as in Fig. 1

### *Phenolic compounds*

The changes in the enzymatic activities and color of the post-culture liquids with RBBR were accompanied by the release of low-molecular phenolic compounds. The concentration of methoxyphenols and hydroxyphenols was generally higher in the cultures of fungi isolated from compost II, i.e. *Trichoderma citrinoviride* strains VI, VIII, XXV, than in the fungal cultures from compost I (PGSF). The concentration of methoxyphenols was nearly 2-fold lower than the concentration of hydroxyphenols (Fig. 5). The concentration of phenolic compounds was significantly higher on days 28 and 32 than at the earlier time points, which indicates progressive biodegradation of RBBR. On the initial culture days, the concentration of these compounds was lower on average by 69.0% and 57.0% in the cultures of fungi isolated from compost I and II, respectively than at the last time point. The level of hydroxyphenols was lower on average by 57.0% and 66.0%, respectively. Concurrently, the levels of hydroxyphenols and methoxyphenols were shown to be significantly negatively correlated with the enzyme activity in the analyzed post-culture liquids (Table 1).

### *pH of post-culture liquids*

Higher pH values of the post-culture liquids were noted in the cultures of fungal strains isolated from compost I (PSSF). They were in the range of 5.98–6.66 (Table 2). In the cultures of compost II strains, the pH of the post-culture liquids was in the range of 5.67–6.38. There was a slight increase in the pH value, i.e. by 10.0% in the compost I cultures and by 7.0% in the fungal cultures from compost II.

## Discussion

Besides basidiomycetes causing white wood rot, microscopic fungi are regarded as one of the most important groups of organisms colonizing lignocellulosic materials and participating in tlhe modification of aromatic compounds (Chamuris et al. 2000; López et al. 2007). Many authors have reported that microscopic fungi can be used for decolorization of lignin-rich pulp and colored wastewater from the textile industry (López et al. 2006, 2007, Rybczyńska and Korniłłowicz-Kowalska 2015).

Although ongoing investigations are focused on enhancement of the efficiency of decolorization and detoxification of colored

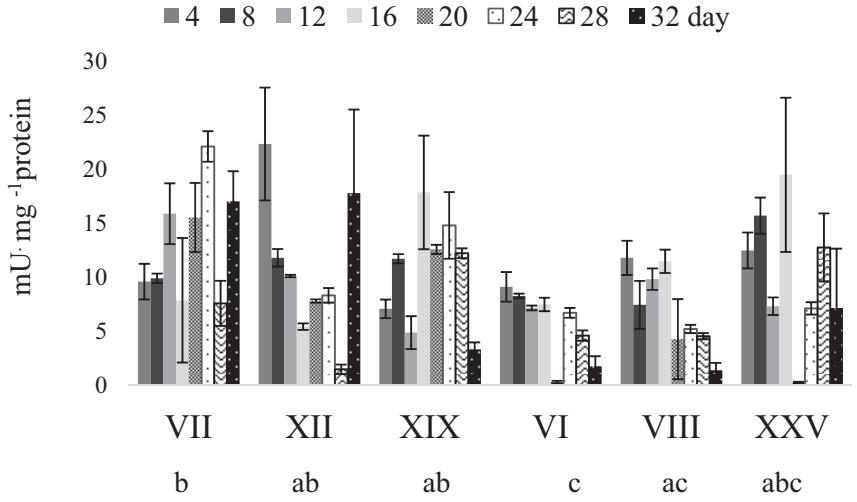


**Fig. 4.** Changes in superoxide dismutase (SOD-like) activity in post-culture liquids of *Trichoderma* strains isolated from composts. Explanations as in Fig. 1

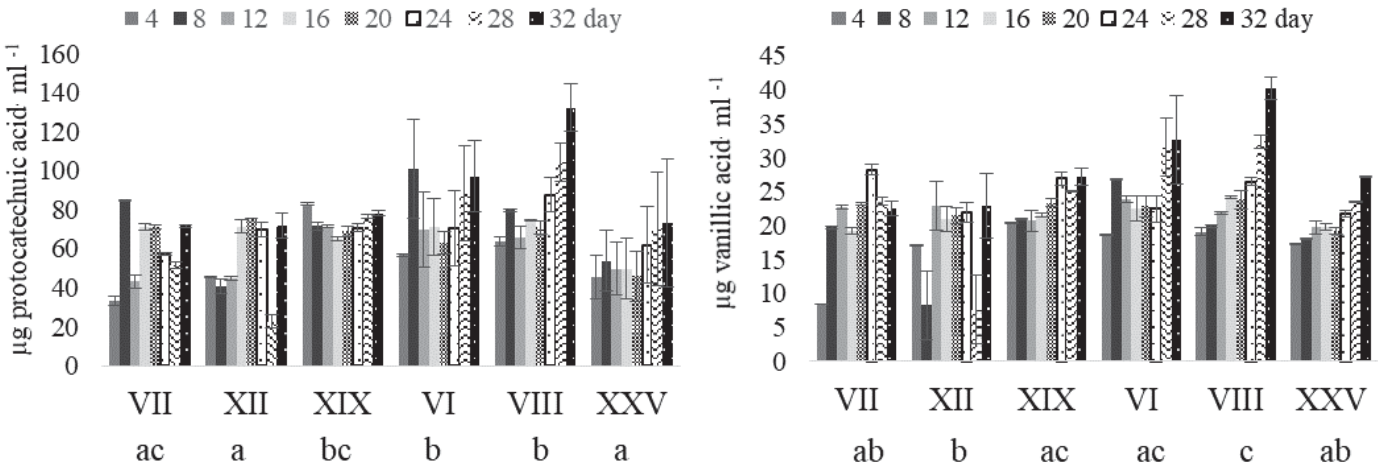


**Fig. 5.** Changes in the concentration of phenolic compounds (with -OH and -$OCH_3$ groups) in cultures of *Trichoderma* strains isolated from composts. Explanations as in Fig. 1

**Table 2.** pH of post-culture liquids

| strains | days of culturing | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 |
| VII | 6.46 ±0.02 | 6.37 ±0.05 | 6.40 ±0,01 | 6.38 ±0.03 | 6.59 ±0.01 | 6.66 ±0.08 | 6.38 ±0.04 | 6.44 ±0.07 |
| XII | 6.02 ±0.01 | 6.30 ±0.04 | 6.41 ±0,02 | 6.45 ±0.03 | 6.47 ±0.05 | 6.50 ±0.05 | 6.65 ±0.06 | 6.48 ±0.03 |
| XIX | 5.98 ±0.02 | 6.04 ±0.02 | 6.23 ±0,01 | 6.20 ±0.01 | 6.19 ±0.02 | 6.26 ±0.03 | 6.17 ±0.02 | 6.15 ±0.02 |
| VI | 5.85 ±0.06 | 5.81 ±0.14 | 6.12 ±0,03 | 6.31 ±0.02 | 6.32 ±0.02 | 6.31 ±0.03 | 6.30 ±0.01 | 6.24 ±0.03 |
| VIII | 6.04 ±0.05 | 5.88 ±0.08 | 5.67 ±0.11 | 5.98 ±0.08 | 6.06 ±0.13 | 6.01 ±0.16 | 6.12 ±0.12 | 6.09 ±0.14 |
| XXV | 6.30 ±0.12 | 6.24 ±0.19 | 6.17 ±0.24 | 6.22 ±0.34 | 6.38 ±0.27 | 6.26 ±0.28 | 6.31 ±0.16 | 6.04 ±0.36 |

industrial wastewater, there are still no data with clear indication of strains with high bioremediation capability and concurrent high adaptability to growth in water environments, especially wastewater.

The fungal strains isolated from the lignocellulosic composts selected in this study for biotransformation of anthraquinone dyes in water environment were characterized by similar and even higher ability to modify Remazol Brilliant Blue R in comparison with white rot fungi (Korniłłowicz-Kowalska i Rybczyńska 2012). This was particularly evident in the case of strains isolated from compost I (PGSF), which had higher content of an easily available carbon and energy source, i.e. grass. Some of these strains removed over 60% of the color produced by 0.02% RBBR. All three *Trichoderma* strains from this compost had RBBR decolorization efficiency in the range of 3.7–62.3%. High decolorization activity of fungal strains isolated from composts was also reported by López et al. (2006). It was reflected by 50–95% loss of color associated with the presence of Remazol Brilliant Blue R, Poly R-478, and Poly S-119. The dye decolorization rate is important in terms of application. Strains characterized by a higher decolorization rate are more promising than strains requiring a longer time to achieve the same effect. The investigated *Trichoderma harzianum* and *T. asperellum* strains isolated from compost I exhibited 0.2 to 27.62% efficiency of 0.02% RBBR decolorization already within the first 8 days. This is consistent with the results reported by Korniłłowicz-Kowalska and Rybczyńska (2012), who found a similar level of decolorization of 0.01% RBBR within the first 7 days of culture carried out by the anamorphic white rot fungus *Bjerkandera adusta* CCCBAS 930. The highest effectiveness of RBBR decolorization in the present study was exhibited by strains isolated from the 30-week-old composts, especially *T. asperellum* XIX, which successively decolorized RBBR along the increase in the culture time. Similar results were obtained by Megha et al. (2015) in their experiments on decolorization of RBBR by *Mucor hiemalis* strain MV04 isolated from an eucalyptus tree. In contrast to the strains isolated from compost I, the *T. citrinoviride* strains isolated from compost II, which contained a greater amount of the hardly available lignocellulose fraction from sawdust (> 51%), decolorized the dye with only slight efficiency. The higher efficiency of decolorization of RBBR, which is a monomeric dye, by the strains isolated from compost I may have been related to their adaptation to degradation of the more easily available lignin fraction contained in grass lignocellulose present in these composts. The intensification of the color of the aqueous solutions of RBBR-containing post-culture liquids suggests a different mechanism of biodegradation of this dye by *T. citrinoviride*. Formation of dark biodegradation products of aromatic compounds is associated with generation of quinones, as indicated by Westermark and Erikson (1974). The authors suggest that the process is associated with laccase and peroxidase activity and release of phenolic compounds. Another explanation of the culture medium darkening during biodegradation of Poly R-478 by a *T. harzianum* strain was proposed by Rybczyńska and Korniłłowicz-Kowalska (2015). The authors suggest that the increase in the intensity of the culture medium color masking the real degree of decolorization is caused by the production of extracellular pigments based on methoxyphenols present in the solution, e.g. guaiacol, catechol, and syringic acid. This may have been the mechanism of the biodegradation of RBBR by *Trichoderma citrinoviride* observed in the present study. This is confirmed by the significant negative correlation between the degree of decolorization and the concentration of phenolic compounds indicating the degradation of RBBR.

Many studies (Rybczyńska-Tkaczyk and Korniłłowicz-Kowalska 2017, Korniłłowicz-Kowalska et al. 2008) on fungal decolorization and biodegradation of colored aromatic compounds, e.g. post-industrial lignin, anthraquinone dyes, or anthracycline antibiotics, have demonstrated that these processes involve changes in the concentration of low-molecular OH and $OCH_3$ phenols. Therefore, in addition to the activity of ligninolytic enzymes, changes in the content of methoxyphenols and hydroxyphenols were analyzed in this study as an indicator of microbial biodegradation of RBBR in aqueous solutions. As reported by Korniłłowicz-Kowalska et al. (2008), decolorization of post-industrial lignin in cultures of the white-rot fungal strain *Bjerkandera adusta* R59 is associated with a decline in the content of methoxyphenols. In the present study, the concentration of methoxyphenols in the

analyzed *Trichoderma* cultures was almost two-fold lower than the concentration of hydroxyphenols, which may indicate the biodegradation of this anthraquinone dye. Paździor-Czochra et al. (2003) have shown that the appearance of protocatechuic acid indicates progressive biotransformation of aromatic compounds, especially lignin. At the end of the fungal culture, the concentration of both phenolic compounds was found to increase.

The formation of these low-molecular weight phenolic compounds is a result of enzymatic catalysis, which involves such oxidoreductive enzymes as peroxidases. As reported by Grönqvist et al. (2005), peroxidases such as horseradish peroxidase (HRP) catalyze the oxidative coupling of phenolic compounds and generation of colored quinones. The results of the present study have demonstrated that the decolorization and recolorization carried out by all the strains is positively correlated with HRP-like activity. This was also shown by Rybczyńska and Korniłłowicz-Kowalska (2015) in their Principal Component Analysis (PCA) of results obtained in cultures of *T. harzianum* and *Haematonectria haematococca* strains during biodegradation of post-industrial lignin.

Vyas and Molitoris (1995) have reported that peroxidases are the main enzymes involved in the process of bio-bleaching i.e. decolorization of RBBR. Biotransformation of aromatic compounds with the involvement of peroxidases is carried out over a wide pH range of 4.00–10.00 (Rybczyńska-Tkaczyk and Korniłłowicz-Kowalska 2017). The pH value used in the present study was in the range of 5.98–6.66. In turn, López at al. (2006) showed a significant correlation between the degree of dye decolorization carried out by groups of compost microorganisms and the production of ligninase (lignin peroxidase), whose optimum pH value is 3–4.5 (Plácido and Capareda, 2015).The present study showed the highest HRP-like peroxidase activity on the first 12 days of the experiment in the cultures of *Trichoderma asperellum* strain XIX, in weeks 16 and 20 in the cultures of *T. citrinoviridae* strains VI, VIII, XXV, and at the end of the experiment, i.e. on days 28 and 32 of the culture of *T. lixii* XII and *T. harzianum* VII. In turn, the xylanase activity in the cultures of all the fungal strains was high until day 12. This was confirmed by the significant negative correlation between the activity of this enzyme and the duration of the experiment. The xylanase activity detected in the fungal cultures run on the RBBR-containing medium may suggest involvement of the enzyme in the transformation of this dye mainly in the initial period. The significant negative correlation between the xylanase and horseradish peroxidase-like activities indicates that, after depletion of the easily available C and energy source, RBBR biodegradation pathways are triggered through an increase in peroxidase activity, and the RBBR dye is thus decolorized (0.329***) especially in the *T. harzianum* and *T. lixii* cultures. A different mechanism of biodegradation may be proposed in the case of the *T. asperellum* culture, as the decline in the HRP-like activity was accompanied by a decrease in the xylanase activity and an increase in the superoxide dismutase (SOD) activity.

Malarczyk et al. (1995) have demonstrated that biodegradation of the lignocellulosic complex is coupled with an increase in superoxide dismutase activity and a simultaneous decrease in the activity of ligninolytic enzymes in peroxidase-producing fungi. This is caused by scavenging of free radicals generated during biodegradation of the lignocellulosic complex. Such a correlation was also shown in the present study, but it was not significant.

The SOD activity in the cultures of two strains *T. asperellum* XIX and *T. harzianum* VII was higher at the end of the experiment, which is consistent with other results of analyses of aqueous extracts of lignocellulosic composts obtained in the final stage of composting (Bohacz 2017). The author suggested that the increase in the activity of this enzyme was caused by coupling of reactions catalyzed by laccase and SOD. This cooperation involves the ability of SOD to scavenge toxic superoxide anion radicals ($O_2^{\cdot-}$) generated during the oxidative depolymerization of lignin units by laccase.

As shown in the literature, *Trichoderma* representatives are cellulolytic and xylanolytic fungi. They are also soft rot wood fungi (Domsch et al. 2007). Both groups of fungal enzymes, i.e. xylanases and ligninolytic enzymes, are involved in the process of bio-bleaching of wood pulp. The involvement of *Trichoderma harzianum* xylanases in this process was reported by Silveira et al. (1999). The present study was focused on the ability of *Trichoderma* strain to produce xylanase, i.e. an enzyme involved in the initial biotransformation of the lignocellulosic complex. Ascomycetes, represented by *Trichoderma* fungi isolated from lignocellulosic composts, are regarded as potential degraders of a number of aromatic compounds (Chamuris et. al. 2000). In cultures of *Coniochaeta ligniaria* ascomycetes, López et al. (2007) determined the activity of xylanase and cellulase on a medium enriched with various C and energy sources from oat spelt xylan, cellulose microcrystalline, and Kraft lignin. They demonstrated that, on the first days of culture, these fungi release active xylanase, but its amount significantly declines after 15 days of culture. The present study showed a reduction of the activity of this enzyme after 12 days of the *Trichoderma* culture. The significant negative correlation between the xylanase activity and the level of phenolic compounds, as in the case of the HRP-like activity, may confirm the involvement of both groups of enzymes in RBBR transformations and the progressing biodegradation process.

## Conclusion

With their high capability of decolorization of Remazol Brilliant Blue R, secretion of oxidoreductive enzymes, and release of low-molecular phenolic compounds, *Trichoderma* fungi, in particular *Trichoderma asperellum* and *T. harzianum*, can catalyze the biodegradation and detoxification of colored aromatic contaminants of anthropogenic origin. Furthermore, their great adaptability to water environments, including wastewater, gives *Trichoderma* fungi an advantage over other fungi in the bioremediation of such environments.

The decolorization of Remazol Brilliant Blue R by the analyzed *Trichoderma* strains increases significantly with the increase in peroxidase activity and is significantly negatively correlated with the generation of low-molecular phenols. The significant negative correlation between the level of these compounds and xylanase activity indicated that the enzyme was involved in the RBBR transformations in the *Trichoderma* cultures. In contrast to HRP-like peroxidase, this effect was observed in the initial culture period.

The dynamics of changes in the horseradish peroxidase-like, superoxide dismutase, and xylanase activities in the aqueous solutions of post-culture liquids from the studied fungal strains depended significantly on the duration of the culture and on the strains.

The differences in the decolorization abilities of the tested *Trichoderma* strains may result not only from the availability of complex organic matter in the analyzed composts, but also from the physiological traits of the fungal species. All *Trichoderma citrinoviride* strains isolated from compost with the higher level of hardly degradable organic matter did not exhibit the ability to decolorize RBBR.

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