# THE ABILITY TO DECOLORIZE DIFFERENT SYNTHETIC DYES DUE TO LACCASE PRODUCED BY *TRAMETES VERSICOLOR* AND *FOMES FOMENTARIUS*

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*Abstract:* The textile industry, by far the most frequent user of synthetic dyes, is in need of ecologically efficient solutions for its colored wastewaters. White-rot basidiomycetes are among the most potent organisms to biodegrade and detoxify a wide range of pollutants and synthetic dyes, enabled by ligninolytic enzymes, namely, laccases. Present study was undertaken to explore white-rot fungi *Trametes versicolor* and *Fomes fomentarius* for their laccase production and potential in dye decolorization. The laccase production was induced by 0.5 mM copper sulphate and their effectity in decolorization of five types of synthetic textile dyes (N-heterocyclic, azo, triphenylmethane and triarylmethane) was studied. The most effective laccase decolorization was observed for the dyes Malachite Green and Bromothymol Blue using 0.5 mM copper as inducer for both fungi.

*Key Words:* Decolorization, copper inducer, laccase, *Trametes versicolor*, *Fomes fomentarius*

## INTRODUCTION

Wastewater from textile industries is characterized by high concentration of chemicals suspended solids and intensively colored aromatic structure due to the extensive use of synthetic dyes and pigments. Based on the chemical structure of the chromophore group, these dyes are classified as azo, anthraquinone, triphenylmethane, heterocyclic and polymeric dyes (Yang et al. 2009).

Typically, these dyes are removed by chemical and physical methods like adsorption, coagulation-flocculation, oxidation, filtration and electrochemical treatments. All these methods have different color removal capabilities, capital costs and operating speed. However, these methods create huge amounts of sludge which become a pollutant on its own creating disposal problems and can cause the formation of toxic carcinogenic and mutagenic breakdown products. There is a great need to develop an economic and effective way of dealing with the textile dyeing waste at the level of the industry itself in the face of the ever increasing production activities.

Biological treatment using white-rot fungi have been demonstrated to be capable of transforming, mineralizing and removing of a wide range of organopollutants, such as polycyclic aromatic hydrocarbons, chlorophenols and polychlorinated biphenyls and various azo, heterocyclic and polymeric dyes due to containing rich ligninolytic enzymatic system. The most important enzyme is laccase (Lac, E.C. 1.10.3.2), which is a multicopper enzyme, which catalyses the oxidation of phenolic and non-phenolic compounds and belongs to the group of phenol oxidases.

However, ligninolytic enzymes from white-rot fungi are only secreted in small amounts, so their using in industrial applications has been limited due to low productivity and high economic cost (Rivera-Hoyos 2013).

Enzyme production can be affected by many factors. One of the most critical factors is type and concentration of the inducer agent (Majeau 2010). The inducer is a specific molecule that induces synthesis of the relevant inducible enzyme and is usually a substrate for a given enzyme. Collins and Dobson (1997) and Palmieri et al. (2000) found that laccase expression of basidiomycetes is induced by copper at a transcriptional level. It is known that the most important metal for white-rot fungi is copper, which is a cofactor in the catalytic center of laccase; thus, a minimum concentration (millimolar range) of copper ions was necessary for production of the active enzyme.

The possible mechanism for this phenomenon is that copper ions enhance the laccase genetic transcription level during the laccase synthesis. A higher enzyme activity guarantees a higher and faster transformation of the target substrate and improves the applicability and effectiveness of the enzyme catalyzed processes (Rao et al. 2014).

In this paper, the most effective laccase produced by *Trametes versicolor* and *Fomes fomentarius* was chosen based on previous study and induced by 0.5 mM copper sulphate and further investigated with respect to the effects on synthetic dye decolorization.

## MATERIAL AND METHODS

### Fungal strains and culture conditions

Locally isolated fungal strains *Trametes versicolor* and *Fomes fomentarius* obtained from the Culture Collection of the Faculty of Forestry and Wood Technology of the Mendel University in Brno (Czech Republic) were used in this study. Cultures were cultivated on Potato Dextrose Agar (PDA) for 10 days at 22 °C.

### Dyes decolorization

The dye decolorization experiment was performed using five different synthetic dyes from different dye classes such as Malachite Green (MG), Crystal Violet (CV), Bromothymol Blue (BB), Methyl Red (MR) and Methylene Blue (MB). Table 1 shows the final concentration and the maximum wavelength of the dyes. The light absorption for each dye was monitored using a VISIONlite SCAN program on Helios Epsilon spectrophotometer.

*Table 1 Concentration and wavelength of synthetic dyes*

| Dyes | Concentration [mg/l] | Wavelength [nm] |
|---|---|---|
| Malachite Green | 7 | 615 |
| Crystal Violet | 20 | 590 |
| Bromothymol Blue | 50 | 605 |
| Methyl Red | 100 | 530 |
| Methylene Blue | 240 | 665 |

### Decolorization of dyes by *Trametes versicolor* and *Fomes fomentarius* fungal culture

For decolorization experiments, three agar plugs (5 $mm^2$) of active mycelium from PDA plates were transferred aseptically into 50 ml Erlenmeyer flasks containing 30 ml of PDB (Potato Dextrose Broth) medium with different concentration of dyes (Table 1) and incubated at 28 °C in dark for 20 days. Decolorization experiment was prepared in different variants: sterilized medium with dyes; sterilized medium with dyes and 0.5 mM copper inducer and sterilized medium containing the dyes but not inoculated with the fungus. Culture samples were collected every 4 days, centrifuged at 10 000 g for 10 min at 4 °C and the supernatants obtained were used for decolorization assay by fungal culture.

### Decolorization assay by fungal culture

Dye concentrations were selected in order to obtain approximately 1.5 absorbance units at the maximum wavelength in the visible spectrum. All the tested flasks were incubated at room temperature, without shaking and in dark. The residual dye concentration was measured spectrophotometrically at their maximum wavelength, as shown in Table 1, and calculated from measured absorbance according to the following expression:

$$\% = \frac{A_0 - A}{A_0} \times 100$$

,

where % is the decoloration percentage obtained, $A_0$ the initial absorbance and $A$ is the final absorbance. A control test containing the same amount of a heat-denatured laccase was also performed in parallel.

## RESULTS AND DISCUSSION

The decolorization of model synthetic dyes is a simple method to assess the bioremediation potential of ligninolytic enzymes, especially laccase. In general, the efficiency of decolorization depends on the structure of dye, fungi and enzymes used and experimental conditions as well as presence of inducer (Zhang et al. 2006). The ability of fungal mycelium obtained from *Trametes versicolor* and *Fomes fomentarius* to decolorize 5 synthetic dyes with different structure was examined. Acquired results were compared with experiment when the fungal laccase was induced by 0.5 mM copper sulphate.

### Day decolorization by two different white-rot fungi

Many studies with *Trametes* strains have been extensively conducted. In our study *Trametes versicolor* and *Fomes fomentarius* were chosen as significant producers of laccase (Rodrigues et al. 2008). Higher effect of dye decolorization was observed using *Trametes*, which agree with observation of Selvam et al. (2002), who used *Fomes* and *Trametes* strains to decolorization of industrial dyes and maximal effect was observed using *Trametes* on the fourth day (Figure 1). The slow increase of decolorization by *Fomes* was observed during all the time of cultivation (Figure 2). Large variations exist among different white-rot species in the ability to produce the different isoenzymes, but also they can vary over time (Hatakka and Hammel 2010). However, Neifar et al. (2011) studied *Fomes fomentarius* and showed a promising future of applying laccase system of this fungus for industrial wastewater decolorization and bioremediation.

### Dye decolorization with and without copper inducer

The positive effect of copper addition in the form of copper sulphate on laccase activity has been reported by many authors (Palmieri et al. 2000, Levin et al. 2002). In our study the copper was used as inducer to increase dye decolorization effect.

Addition of copper sulphate stimulated decolorization, the presence of copper increased around 3-4-fold the degradation capability by both fungi throughout the experiment in comparison with cultivation without laccase inducer.

The higher decolorization effect using copper suggested that induction of laccase significantly influenced decolorization process. It is known that the presence of laccase inducer ($Cu^{2+}$) in the process increased the range and rate of decolorization (Levin et al. 2002).

Our finding is in accordance with work of Zouari-Mechichi et al. (2006), who observed that the decolorization using *Trametes trogii* is possible in the presence of $Cu^{2+}$ and it was faster when $Cu^{2+}$ was present in the medium. Lorenzo et al. (2006) observed that addition of copper increased laccase activity and this factor played an important role in the decolorization of the textile dye.

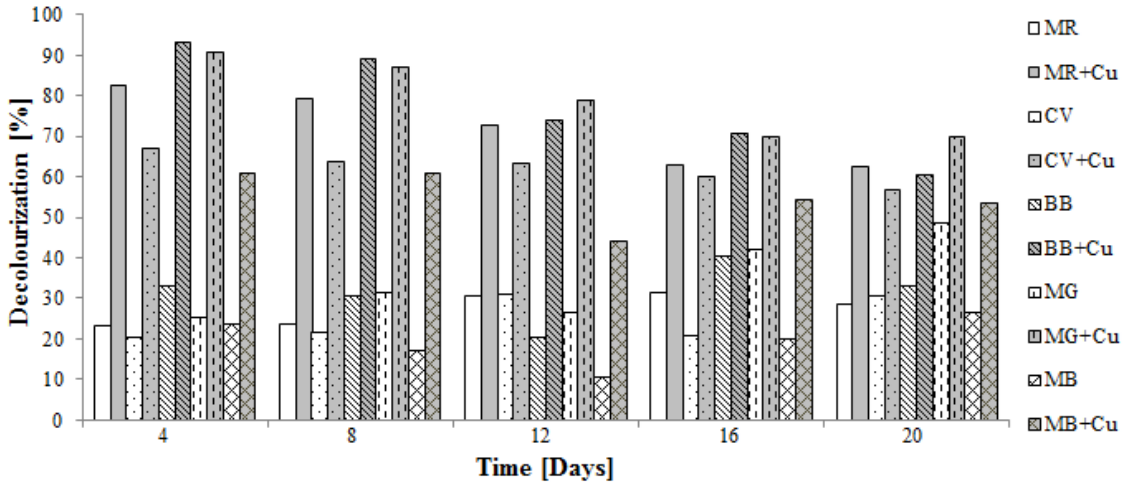
### Decolorization effect of five types of synthetic dyes

The synthetic dye decolorization efficiency of the biomass from fungus *Trametes versicolor* was the highest for the triarylmethanic dyes Malachite Green and Bromothymol Blue with a maximum of 95% after 4 days of cultivation using 0.5 mM copper inducer. For *Fomes fomentarius* the ability to decolorize the triarylmethanic dyes around 70% during the period of cultivation was observed. Bromothymol Blue and Malachite Green share very close structure with three benzene rings and these dyes might fit well with the enzyme activity centers and can be degraded (Ling et al. 2015). It is in contrary with work of (Jayasinghe et al. 2008), who tested different fungal strains and *Fomes fomentarius* showed better mycelial growth and decolorization of Malachite Green than *Trametes versicolor*.

The decolorization ability for Methyl Red, which belongs to azo dyes, was around 80% for both fungal strains, which agree with work of Sharma et al. (2015), who tested ability of white-rot fungi to decolorize azo dyes. Wong and Yu (1999) reported that dye decolorization by fungi was dependent on dye structure. Different dyes have different molecular structures. So a fungus capable of decolorizing

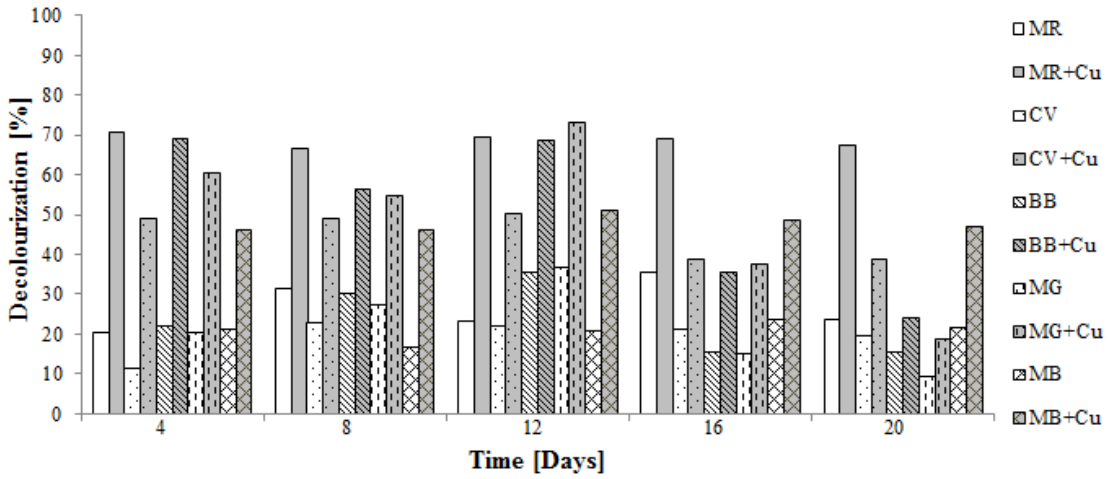
one dye may have different capacities for other dyes. One of the lowest decolorization was observed for Crystal Violet, which belongs to triphenylmethane dye and for heterocyclic dye Methylene Blue. It can be due to the fact that the triphenylmethane dyes are known to be resistant to enzymatic decolorization in comparison with azo dyes and hence more time for decolorization is required (Hughes and Poole 1991). It is in contrast with the study Yan et al. (2009), who suggested the efficient decolorization of Crystal Violet using fungus *Trametes trogii*.

*Figure 1 The synthetic dye decolorization of fungal mycelium from Trametes versicolor with and without copper*




*Legend: MR – Methyl Red, MR+Cu – Methyl Red + copper, CV – Crystal Violet, CV+Cu – Crystal Violet + copper, BB – Bromothymol Blue, BB+Cu – Bromothymol Blue + copper, MG – Malachite Green, MG+Cu – Malachite Green + copper, MB – Methylene Blue, MB+Cu – Methylene Blue + copper, standard deviation is less than 0.5%*

*Figure 2 The synthetic dye decolorization of fungal mycelium from Fomes fomentarius with and without copper*




*Legend: Legend: MR – Methyl Red, MR+Cu – Methyl Red + copper, CV – Crystal Violet, CV+Cu – Crystal Violet + copper, BB – Bromothymol Blue, BB+Cu – Bromothymol Blue + copper, MG – Malachite Green, MG+Cu – Malachite Green + copper, MB – Methylene Blue, MB+Cu – Methylene Blue + copper, standard deviation is less than 0.5%*

The mechanism for decolorization by white-rot fungi consist of a combination of biosorption by fungal mycelia and biodegradation by extracellular laccase. High decolorization effect by fungal mycelium is through adsorption of the dyes onto its cell surface. This type of decolorization has been reported to be the primary mechanism of decolorization and major to decolorization by enzymatic preparations (Selvam et al. 2002).

## CONCLUSION

In this study white-rot fungi *Trametes versicolor* and *Fomes fomentarius* have been investigated as potential producers of laccase enzyme and copper sulphate was successfully used as potential inducer of laccase. Fungi were used to decolorize five synthetic dyes in the form of fungal mycelium. This effect is more pronounced, when copper inducer was present in cultivation medium. Therefore, laccase inducer showed great potential to be used in process of color removing from textile wastewaters.

Higher decolorization effect was observed using *Trametes* strain than *Fomes* and our study confirmed that each strain has different capability to decolorize synthetic dyes.

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