

Evaluation of *Pleurotus ostreatus*, *P. sajor-caju* and *Ganoderma lucidum* Isolated from Nature for their Ability to Decolorize Synazol Blue and Synazol Red Textile Dyes

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Biological decolorization of Synazol Blue and Synazol Red were comparatively studied using three different wild-type white-rot fungi strains (*Pleurotus ostreatus*, *P. sajor-caju* and *Ganoderma lucidum*) isolated from nature. The initial dye concentrations in the medium were 250, 500 and 1000 mg/L, respectively. All the organisms studied decolorized Synazol Blue to varying degrees. At low dye concentration, fungi strains resulted in the best decolorization, but mycelia negatively affected from increasing dye concentrations.

Key words: White-rot fungi, Biological decolorization, textile dye.

Recently, new and tighter regulations coupled with increased enforcement concerning wastewater discharges have been forced in many countries. This tight legislation, in conjunction with international trade pressures, such as increasing competition and the introduction of eco-labels for textile products on the European and US markets, has been threatening the very survival of the textile industry in many industrialized countries.

Commonly applied treatment methods for colour removal from coloured effluents consist of integrated processes involving various combinations of biological, physical and chemical decolorization methods^{1, 2, 3}. These integrated

treatment methods have limited efficiency and suffered from several drawbacks such as high amounts of chemical usage and/or sludge generation, costly infrastructure requirements and/or high operating expenses. Conventional wastewater treatment plants relying on activated sludge systems are not adequate for the treatment of textile mill effluents, since the use of bacteria in the biological treatment of dye effluents may result in the generation of colourless, dead-end aromatic amines, which are generally more toxic than the parent compounds⁴. In view of the need for a technically and economically satisfying treatment technology, a flurry of emerging technologies (e.g. biological processes, granular activated carbon filtration, foam floatation, electrolysis, photocatalysis, biosorption and Fenton oxidation) are being proposed and tested at different stages of commercialization⁵.

By far, the single class of microorganisms that is most efficient in breaking down synthetic dyes is the white rot fungi^{6, 7}. Most these fungi, especially *Pleurotus* species, are robust organisms

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and are generally more tolerant to high concentrations of polluting chemicals than bacteria^{8, 9}. These fungi mostly belonging to basidiomycetes are capable of extensive aerobic lignin depolymerization and mineralization in nature. This property is claimed to be based on the capacity of white rot fungi to produce one or more extracellular lignin-modifying enzymes⁷. The white rot fungi, including *Pleurotus* species, have been reported on several occasions as good producers of extracellular ligninolytic enzymes and as active strains for textile dye decolorization and other pollutants^{10, 11}. Nevertheless, to the best of our knowledge, no information is available regarding the use of *Pleurotus ostreatus*, *P. sajor-caju* and *Ganoderma lucidum* for the decolorization of most the widely used dyes Synazol Blue and Synazol Red among textile producers. This study, in which three strains of white-rot fungi were examined for the decolorization of Synazol Blue and Synazol Red. The objective of the present study was to comparatively evaluate the potential of *Pleurotus ostreatus*, *P. sajor-caju* and *Ganoderma lucidum* to degrade Synazol Blue and Synazol Red and to estimate the longevity and sustainability of the fungus under toxic conditions.

MATERIALS AND METHODS

The decolorization study was carried out with three strains of white-rot fungi (*Pleurotus ostreatus* MCC07, *P. sajor-caju* MCC29, *Ganoderma lucidum* MCC52). Wild type strains were isolated from the different parts of Anatolia. All the strains were deposited in the Mushroom Culture Collection (MCC) of the Department of Biology, Faculty of Science and Letters, Celal Bayar University, Turkey and maintained on malt extract agar slants at 4°C until use.

All the strains were inoculated on agar plates (90 mm in diameter, 20 ml medium/Petri-dish) containing modified Kirk's basal salt media with the following composition: glucose 1.0 g, urea 0.036 g, KH_2PO_4 2.0 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g, CaCl_2 0.099 g, agar 20.0 g in 1000 ml distilled water. Agar plates were supplemented with Synazol Blue and Synazol Red corresponding to dye concentrations of 250, 500 and 1000 mg/L. Inoculums consisted of 6 mm agar plugs of one week old cultures grown on modified Kirk's basal medium at 27°C. The plug cut

under sterile conditions from the outer edge of the agar plate was transferred onto the centre of the experimental plates for each replicate. In addition, non-inoculated plates served as controls for abiotic decolorization⁵. Each fungus was tested in three independent experiments on all plates. The plates were incubated at 27°C for at least 30 days. Mycelial growth was followed by measuring radial extension of the mycelium as described by Weitz et al.¹² with a caliper gauge along two diameters at right angles to one another and the average diameter for each plate calculated. The mean mycelial growth was then calculated from the three replicates of each treatment. A decolorized zone appeared when the fungus degraded the dye.

Also, mycelial plugs (diameter 6 mm) were used as inoculum for spectrophotometric analyses of decolorization period. Erlenmeyer flasks containing 50 ml of liquid Kirk's basal media and textile dyes (Synazol Blue and Synazol Red). The dye concentrations chosen were 250, 500 and 1000 mg/L because dyehouse effluents typically contain 600-800 mg/L dye¹³. Liquid media were sealed by cotton plugs and autoclaved at 121°C for 15 min. They were kept in shaker incubator at 27°C, 120 rpm/min. Decolorization of textile dyes in the culture medium were monitored at regular intervals during the experimental study.

Liquid samples (0.5 ml) were taken from each reaction flask at regular time intervals, and residual color was measured immediately by a UV-vis spectrophotometer () at the maximum wavelength of absorbance (610 nm). Absorbance values were used for the calculations of decolorization efficiencies. Distilled water containing Kirk's basal medium was used as reference⁵. The data presented are the averages of the results of three replicates with a standard error of less than 5%.

RESULTS AND DISCUSSION

Reactive dyes are the most commonly used dyes in the textile industry. Synazol Blue and Synazol Red are widely preferred reactive dyes, were used as dyes for the determination of decolorization potential of three white-rot fungi strains, in this study.

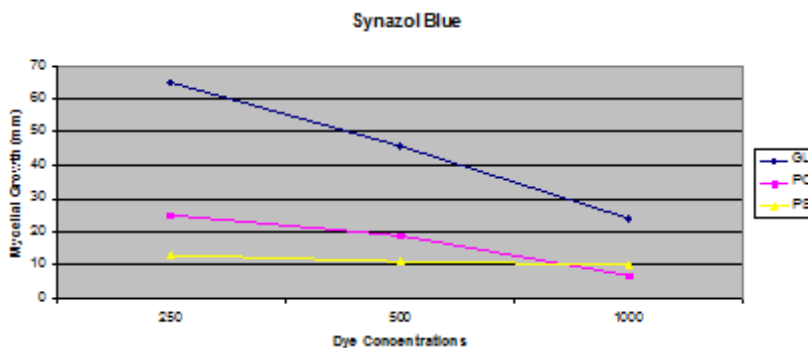
The effect of dye concentrations on the growth of organisms studied is depicted in Figure

1 and 2. All organisms were negatively affected from an increasing amount of dye in growth media, resulting in significantly lower mycelial growth. Increasing concentrations of Synazol Blue and Synazol Red in the growth medium caused up to 3 and 12 times lower mycelial growth for all organisms used, respectively (Figure 1, 2). Similar results have been reported in literature in which amaranth dye was used. It has been reported that 100 mg of amaranth dye in Kirk's basal salt medium containing 1 g of glucose shows a more toxic effect on the growth of *T. versicolor* than a lower amaranth concentration of 33 mg in the same medium¹⁴.

As depicted in Figure 1 and 2, even though the mycelial growth was retarded when the organisms were exposed to increasing dye concentrations, the decolorization efficiencies of the organisms were not deteriorated that much for Synazol Blue (Figure 3). Figure 3 and Figure 4, depict the color removal efficiency of each organism used at 250, 500 and 1000 mg/L dye concentrations, respectively. As it can be seen in Figure 4,

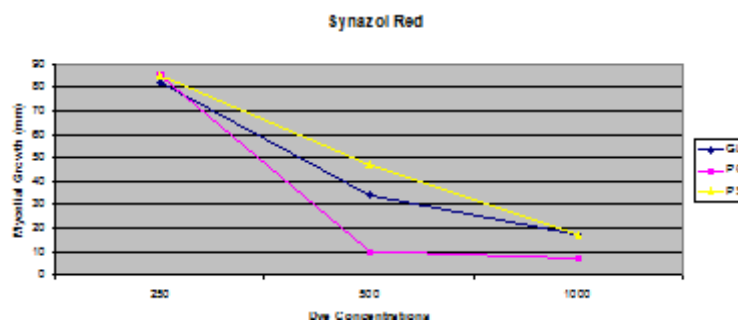
increasing concentration of Synazol Red negatively affected the decolorization efficiency of the organisms to varying degrees. Organisms could be enumerated as to their decolorization efficiencies when they were exposed to 500 mg/L dye concentration in the growth media for Synazol Blue as follows: *G. lucidum* = *P. ostreatus* (98%) > *P. sajor-caju* (97%), and for Synazol Red as follows: *P. sajor-caju* (8%) > *G. lucidum* (2%) > *P. ostreatus* (1%), respectively. A similar order could be given when the organisms were exposed to 1000 mg/L dye concentrations as follows: for Synazol Blue *P. ostreatus* (97%) > *G. lucidum* = *P. sajor-caju* (96%), and for Synazol Red *P. sajor-caju* (2%) > *G. lucidum* = *P. ostreatus* (1%), respectively. The decolorization efficiency of all organisms decreased in general for Synazol Red textile dye.

It has been reported in the literature that several textile dyes are aerobically biotransformed or mineralized by the white-rot fungi *Phanerochaete chrysosporium*, *T. versicolor*, *P. ostreatus*, etc.^{1,2,6,15}. As compared with the many



GL: *Ganoderma lucidum*; PO: *Pleurotus ostreatus*; PS: *Pleurotus sajor-caju*

Fig. 1. Effect of various Synazol Blue concentrations on mycelial growth at 23rd day



GL: *Ganoderma lucidum*; PO: *Pleurotus ostreatus*; PS: *Pleurotus sajor-caju*

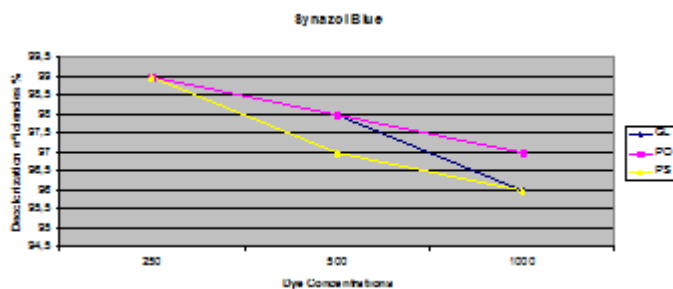
Fig. 2. Effect of various Synazol Red concentrations on mycelial growth at 23rd day

literature reports, we were able to decolorize significantly higher concentrations of dye (250, 500 and 1000 mg/L) in our study. In a recent study by Reife and Freeman¹⁶, it was indicated that azo dyes, owing to their chemical structure, are more resistant to decolorization than are anthraquinone dyes. It was found that *P. ostreatus* wild-type strains are the most robust species, since the drop in decolorization efficiency due to the increased dye concentration was around 4%-14%, while the other organisms were significantly affected drop in decolorization efficiency: *P. ostreatus*=60%, *P. djamor*= 34%, and *P. citrinopileatus*=19%. These findings are in correspondence to the recent studies related to *Pleurotus sp.*^{3, 10, 15, 17}. Yesilada et al.¹⁸ reported that *P. ostreatus* shows 97%, 89%, and 84% decolorization for azo dyes (264 mg/L), namely, Astrazone Red, Astrazone Blue and Astrazone Black, respectively. The white-rot basidiomycetes *Phanerochaete chrysosporium* is also a well-known organism that is able decolorize textile dye effluents. Different decolorization levels (40%-73%) are achieved for 8 textile dyes in Kirk's basal medium by this organism⁷.

Dye molecules have many different and complicated structures influencing fungal

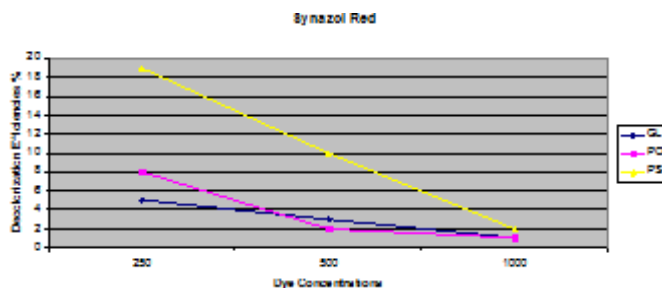
decolorization and the decolorization is a function of dye type. White rot fungi (especially *Pleurotus sp.*) are the only organisms that can more efficiently degrade polymeric components to their monomeric subunits. White rot fungi are also unique organisms that are directly responsible for the oxidative depolymerization of aromatic macromolecules. Fungi may be exposed to a wide variety of organic and inorganic pollutants in the environment, thus, it is obviously desirable that more is known about the impact of pollutants on these organisms. Unfortunately, while it is easy to speculate on the likely effects of pollutants on fungi (production of new and robust extracellular enzymes under unusual environments), it is often far more difficult to demonstrate such effects. Another problem is that it is unlikely that a meaningful picture of how fungi respond to pollutants in the environment can be gained from determining responses to pollutants added to growth media in laboratory experiments. The effects of toxic materials on fungi growing in vitro, for example, are markedly influenced by the composition of the medium used.

In this study, three different white-rot fungi strains were used to reveal the decolorization



GL: *Ganoderma lucidum*; PO: *Pleurotus ostreatus*; PS: *Pleurotus sajor-caju*

Fig. 3. Decolorization efficiencies for each fungus species for Synazol Blue at 23rd day



GL: *Ganoderma lucidum*; PO: *Pleurotus ostreatus*; PS: *Pleurotus sajor-caju*

Fig. 4. Decolorization efficiencies for each fungus species for Synazol Red at 23rd day

potential of Synazol Blue and Synazol Red textile dyes. A fungus capable of decolorizing one dye has different capacities for other dyes. There is a need to determine fungal strains that are capable of decolorizing dye wastewater and the inhibitory effects of dyes on fungal growth. Our findings could contribute to a better knowledge of the decolorization abilities of three different white-rot fungi strains (*P. ostreatus*, *P. sajor-caju*, and *Ganoderma lucidum*) for Synazol Blue and Synazol Red, which has not been studied in detail up to now. Results emerging from this study provide a background useful to propose new eco-friendly alternatives for the wastewater treatment of textile industries. This study demonstrates the decolorization potential of wild fungus isolated from nature.

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