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Full Length Research Paper

Evaluation of wild and commercial types of *Pleurotus* strains for their ability to decolorize cibacron black W-NN textile dye

Halil Bıyık[1], Fatih Kalyoncu[2]*, Erman Oryasin[1], Nuri Azbar[3], Erbil Kalmış[3] and Gamze Başbülbül[1]

[1]Adnan Menderes University, Faculty of Science and Arts, Department of Biology, Aydın / Turkey.
[2]Celal Bayar University, Faculty of Science and Arts, Department of Biology, Manisa / Turkey.
[3]Ege University, Faculty of Engineering, Department of Bioengineering, Bornova, İzmir / Turkey.

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Biological decolorisation of Cibacron Black W-NN, was comparatively studied using five commercial (*Pleurotus djamor*, *P. ostreatus*, *P. citrinopileatus*, *P. eryngii* and *P. sajor-caju*) and three wild types of (*Pleurotus ostreatus*, *P. ostreatus* and *P. eryngii*) white rot fungi strains on agar plates. The initial dye concentrations in the medium were 20, 50, 100, 200 and 500 mg/L, respectively. All *P. ostreatus* strains and *P. sajor-caju* fully decolorized Cibacron Black W-NN without any problem. Other organisms were found to be negatively affected from increasing dye concentrations and were able to decolorize the dye used up to a certain concentration (50 mg/L for *P. citrinopileatus*; 100 mg/L for commercial *P. eryngii* and 200 mg/L for wild *P. eryngii*).

Key words: Agar plate screening, biological decolorisation, *Pleurotus spp.*, textile dye.

INTRODUCTION

Synthetic dyes are extensively used in a number of Industries, such as textile dyeing or paper printing. The treatment of wastewater from textile and dyestuff Industries is one of the most challenging. 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 color removal from colored effluents consist of integrated processes involving various combinations of biological, physical and chemical decolorisation methods (Galindo and Kalt, 1999; Robinson et al., 2001; Azbar et al., 2004). 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 colorless, dead-end aromatic amines, which are generally more toxic than the parent compounds (Banat et al., 1996) therefore, may have poor adaptability and 35 limited application to a wide range of dye wastewater (Kulla et al., 1983). In view of the need for a technically and economically satisfying treatment technology, a flurry of emerging technologies (example, biological processes, granular activated carbon filtration, foam floatation, electrolysis, photocatalysis, biosorption 1and Fenton oxidation) are being proposed and tested at different stages of commercialization (Kalmış et al., 2008).

By far, the single class of microorganisms that is most efficient in breaking down synthetic dyes is the white rot fungi (Balan and Monteiro, 2001; Wesenberg et al., 2003). Most these fungi, especially *Pleurotus* species, are robust organisms and are generally more tolerant to

*Corresponding author. E-mail: fatihkalyoncu@hotmail.com. Fax: +902362412158.

high concentrations of polluting chemicals than bacteria (Hou et al., 2004; Levin et al., 2004). 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 enzymes (Wesenberg et al., 2003). 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 decolorisation and other pollutants (Fu and Viraraghavan, 2001; Yonni et al., 2004; Nilsson et al., 2006; Zhao and Hardin, 2007). Nevertheless, to the best of our knowledge, no information is available regarding the use of *Pleurotus* species for the decolorisation of most the widely used dye Cibacron Black W-NN among textile producers. This study, in which eight strains of *Pleurotus spp*. were examined for the decolorisation of Cibacron Black W-NN, is part of a research project that focused on the determination of the decolorisation potential of fungi isolated from nature. The objective of the present study was to comparatively evaluate the potential of both wild and commercial type of *Pleurotus spp* to degrade Cibacron Black W-NN and to estimate the longetivity and sustainability of the fungus under toxic conditions.

MATERIALS AND METHODS

The decolorisation study was carried out with eight strains of *Pleurotus* species (three wild type strains: *Pleurotus ostreatus* MCC007, *P. ostreatus* MCC020, *Pleurotus eryngii* MCC026 and five commercially available strains: *Pleurotus djamor* MCC015, *P. ostreatus* MCC016, *Pleurotus citrinopileatus* MCC023, *P. eryngii* MCC025 and *Pleurotus sajor-caju* MCC029). The commercial strains were obtained from local spawn producers, Agromycel Company (MCC015, MCC023, MCC025 and MCC029) and Slyvan company (MCC016) in Turkey. Wild type strains were isolated from the different parts of Anatolia (MCC007: Burdur; MCC020: Muğla and MCC026: Kars) in the context of another TUBITAK research project (project code: 104T236). All the strains were deposited in the Mushroom Culture Collection (MCC) of the Department of Bioengineering, Faculty of Engineering, Ege 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, $MgSO_4.7H_2O$ 0.5 g, $CaCl_2$ 0.099 g, agar 20.0 g in 1000 ml distilled water. Agar plates were supplemented with Cibacron Black W-NN corresponding to dye concentrations of 20, 50, 100, 200 and 500 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. A control plate with no dye added was also inoculated with each strain. In addition, non-inoculated plates served as controls for abiotic decolorisation.

Each fungus was tested in five 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. (2001) 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. pH of the media was adjusted to 5.4 by using either diluted HCl or NaOH (Kalmış et al., 2007).

The data presented are the averages of the results of three replicates with a standard error of less than 5%.

RESULTS AND DISCUSSION

The effect of initial dye concentration is an important factor in deciding the extent of decolorisation and the growth. MCC007, MCC016, MCC020 and MCC029 fully decolorized Cibacron Black W-NN without any problem. Other organisms were found to be negatively affected from increasing dye concentrations and were able to decolorize the dye used up to a certain concentration (50 mg/L for MCC023; 100 mg/L for MCC025 and 200 mg/L MCC026 respectively) (Table 1). In respect to growth under increasing dye effect, almost all organisms, expect for MCC023, well tolerated the dye existence in growth media and showed almost identical growth rate with the control one (no dye).

The effects of dye concentrations on the growth of fungal organisms studied are depicted in Figure 1 also shades light on the growth of organisms studied under the effect of increasing concentrations of dyestuff used in this study.

All organisms exposed to Cibacron Black W-NN textile dye were able to show a complete growth on the agar plates at all dye concentrations studied at the end of the study. A little retardation in mycelium growth was observed for *P. citrinopileatus* (MCC023), *P. djamor* (MCC015) and *P. eryngii* (MCC025 and MCC026) at 200 and 500 mg/L, although there was no significant inhibition in growth with dye concentrations used. *P. citrinopileatus* was the slowest growing organism at all dye concentrations. *P. ostreatus* strains (MCC007, MCC016 and MCC020) were more active and faster growing organisms among the others. All the organisms, except for MCC023, almost completed their growth in less than 15 days at all dye concentrations.

It has been reported in the literature that several azo dyes are aerobically biotransformed or mineralized by the white rot fungi *Phanerochaete chrysosporium*, *Trametes versicolor*, *Pleurotus ostreatus*, etc. (Galindo and Kalt, 1999; Robinson et al., 2001; Balan and Monteiro, 2001; Eichlerova et al., 2006). Yesilada et al., (2003) 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 eight textile dyes in Kirk's basal medium by this organism.

Researchers found that decolorization efficiency is better under stationary (71.3%) conditions than with agitated

Table 1. Agar-plate screening for decolorisation.

Decolorisation at 10th day of the study					
	Dye Concentrations (mg / L)				
Organisms	**20**	**50**	**100**	**200**	**500**
P. citrinopileatus (MCC-023)	xx	x	O	O	O
P. djamor (MCC-015)	xx	x	x	O	O
P. eryngii (MCC-025)	xx	xx	x	O	O
P. eryngii (MCC-026)	xx	xx	x	x	O
P. sajor-caju (MCC-029)	xxx	xxx	xxx	xxx	xxx
P. ostreatus (MCC-007)	xxx	xxx	xxx	xxx	xxx
P. ostreatus (MCC-016)	xxx	xxx	xxx	xxx	xxx
P. ostreatus (MCC-020)	xxx	xxx	xxx	xxx	xxx

X: Slightly decolorisation, XX: Visible decolorisation observed, XXX: Full decolorisation, O: No decolorisation.

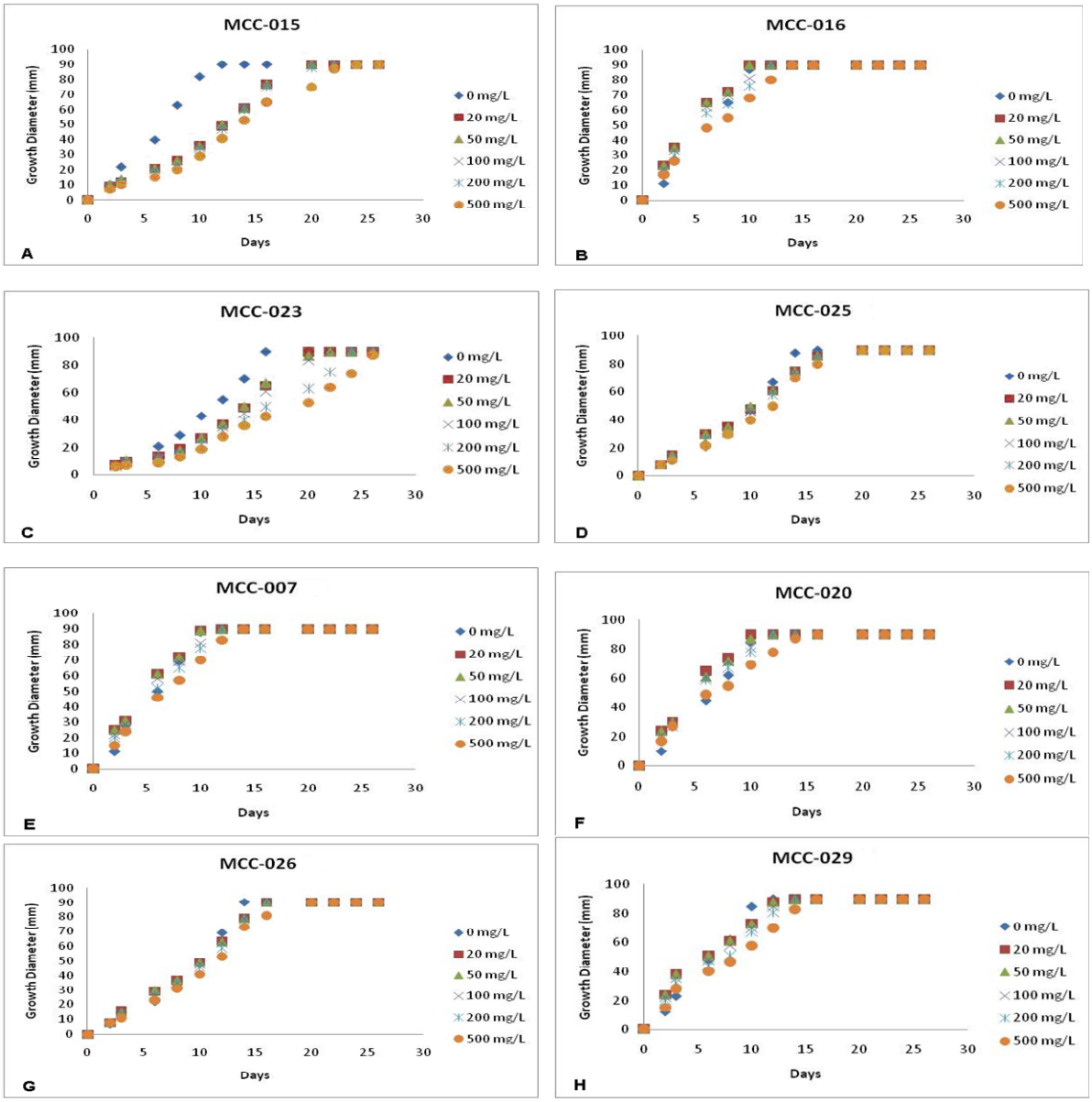

Figure 1 (A - H). The effect of dye concentrations on the growth of organisms studied.
P. djamor (MCC015), *P. ostreatus* (MCC016), *P. citrinopileatus* (MCC023),
P. eryngii (MCC025), *P. ostreatus* (MCC007), *P. ostreatus* (MCC020),
P. eryngii (MCC026), *P. sajor-caju* (MCC029).

agitated cultures (57.2%) and the best decolorization efficiency (78%) is obtained when the combination of the two conditions is employed (Wesenberg et al., 2003). Kalmış et al., (2008) reported that wild type of two *Pleurotus ostreatus* strains (MCC020 and MCC007) showed 83.6 and 81.8% decolorization for Benazol Black ZN textile dye (1000 mg/L). According to Tychanowicz et al. (2004) varying degrees of decolorisation determined on some industrial dyes by *Pleurotus pulmonarius*. In a recent study by Kalmış et al. (2007), it was found that five *Pleurotus* species were able to decolorize for five different textile dyestuffs namely Indanthren yellow F3GC Collosiol, Blue CC Dranix, Indanthren blue CLF Collosiol, Remazol Brilliant blue BB and Levafix Brilliant blue E-B on agar plates.

Dye molecules have many different and complicated structures influencing fungal decolorisation and the decolorisation is a function of dye type. White rot fungi (especially *Pleurotus spp*.) 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, eight different *Pleurotus* strains, of which the three wild types were reported in this research for the first time, were used to reveal the decolorisation potential of Cibacron Black W-NN textile dye. 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 decolorisation abilities of eight different *Pleurotus* strains for Cibacron Black W-NN, 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 decolorisation potential of wild fungus isolated from nature.

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