Textile Dye Dissociation Ability by Selected *Polyporus species*

T.K. Wickremasinghe *and* K.B. Wijesekara
Uva Wellassa University, Badulla, Sri Lanka.

Introduction

The textile industry, by far the highest user of synthetic dyes, is in need of ecologically efficient solutions for its colored effluents. Effluents from textile industries are a complex mixture of many polluting substances such as heavy metals, organochlorine-based pesticides, pigments and dyes. The wastewater containing dyes are highly colored which can cause water pollution, because these dyes are mutagenic, carcinogenic, and also cannot be completely removed by conventional wastewater treatment systems. Therefore, dye-containing effluents should be treated before disposal and discharge to remove or reduce toxic substances. Wood decomposing basidiomycetes fungi are well known for their natural ability to decompose lignin, a highly complex non-phenolic polymer, which also gives them the potential capacity to degrade a wide variety of complex organopollutants. This degradative ability of wood decomposing fungi has opened up new prospects for the development of biotechnological processes to treat textile dye effluents (Lopez et al., 2002). One promising strategy is the use of white-rot fungal and strains that possess the ability to decolorize synthetic dyes (Ferreira et al., 2000). This study investigates the synthetic dye decolorizing ability of some selected *Polyporus* species to identify the potential candidate fungal species to treat dye containing effluents from textile industries.

Methodology

Media preparation, culturing of basidiocarps and fungal culture maintenance: the Potato Dextrose Agar (PDA) medium that included extract of 200 g of Potatoes, 20 g of Dextrose and 15 g of Agar, was used for the culturing and maintaining of *Polyporus* fungal strains. Prepared PDA medium was then sterilized by autoclaving at 1 atm and 121 ^{0} C for 15-20 minutes, supplemented with 0.5 ppm fiter-sterilized solution of Amphicilliene and finally poured into 9.5 cm glass Petri dishes so each of the dishes contained about 30 ml of medium. Fresh basidiocarps of *Polyporussp.* that are associated with decaying wood were collected from in and around Badulla area and brought to the laboratory in sealed polythene bags. Collected fungal basidiocarps were surfaced washed with tap water followed by three washings with sterile distilled water and finally with 70% ethanol to remove dirt. Next, 5mm x 5mm pieces were cut from the actively dividing regions of the basidiocarp, cultured separately in pre-prepared PDA plates and incubated at room temperature and at darkness for three days. Identification of *Polyporussp*was done by studying the morphology of the bacidiocarp and muceliumpyphae under the light microscope. Stock cultures were prepared from well isolated fungal colonies and used in all experiments.

Screening for dye decolourisation ability by *Polyporus sp.:* Initial screening of *Polyporus sp.* was carried out by placing a fungal disc of 5 mm diameter, taken from the actively growing edge of 7 weeks old cultures, on the surface of PDA plates supplemented with 50 ppm of either Malachite green or Bromophenol blue. Culture plates were incubated at room temperature and decolorization was recorded for three days. Ten different *Polyporus sp.* were tested for their dye decolorization ability against each dye type. Two fungal species that demonstrated the highest dye decolorization ability for each dye were selected for the successive quantitative decolorization assay.

Quantitative dye decolourization assay: the decolorization ability of the selected fungal species after the initial screening was quantitatively evaluated in Malt Extracts (ME) liquid medium containing Malachite green or Bromophenol blue, separately. The medium was supplemented with 50, 100, 200 and 400 ppm of each of the dye separately and the final pH was adjusted to 4. Prepared media were dispatched to 100 ml conical flaks each containing 25 ml of medium. Five fungal dices of 5 mm in diameter cut from seven days old cultures were introduced to each of the conical flak and fitted with a cotton plug. Next, flasks were placed on an orbital shaker rotating at 200 rmp and incubated at room temperature for five days. A sample of 2 ml was removed into 2 ml eppendorf tubes separately from each of the flask, at 24 h time intervals. Removed samples were centrifuged using a bench top microcentrifuger at 10,000×g at 4 C. The supernatants were analyzed by measuring the decrease in absorbance at the absorbance maxima (λmax) using a UV spectrophotometer. Dye decolorization ability was determined according to the methods after Sayan (2006) using the equation;

$$\% \text{ Decolourization} = (1-C/C_0) \times 100$$

Where, C_0 is the absorbance of the dye before decolourization and C is the absorption of the dye after decolourization at each sampling time. All the experiments were repeated three times and each treatment within an experiment had three replicates.

Determination of Laccase enzyme activity: laccase enzyme activity in the sample was spectrophotometrically determined by monitoring the rate of formation of dark green colour (product) due to the enzymatic oxidation of the substrate ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) according to the methods describes by Niku-Paavola*et al.* (1988). A solution of 0.5 ml of 0.45 mM ABTS was prepared in 0.1M Na PO . Using a 4 days old culture containing 100 ppm dye, 0.5 ml of laccase enzyme was extracted and mixed with ABTS solution in a 3 ml cuvette. The final volume of the reaction mixture was adjusted to 1 ml using the buffer for ABTS. The kinetic reaction was spectrophotometrically measured at 420 nm as an increase in absorbance, after 1 min reaction time at room temperature.

Statistical analysis: Measurements on absorbance of each fungus type were analyzed using one way ANOVA with MINITAB statistical package version 15.0. Experiment was done using 2x2 Factorials in Complete Randomized Design, for each dye separately, taking fifth day spectrophotometric data.

Results and discussion

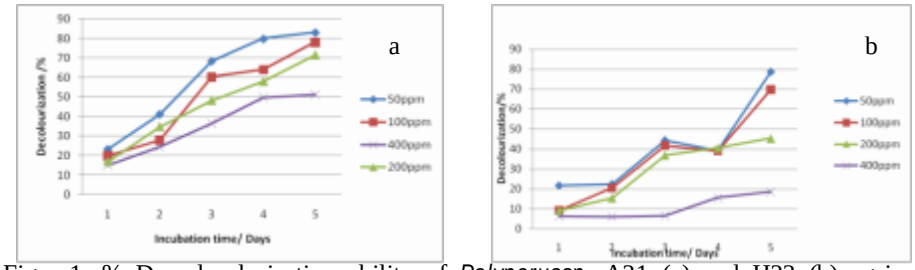

Figure1. % Dye decolorization ability of *Polyporussp*. A21 (a) and H22 (b) against Malachite green.

Out of the ten *Polyporus* species screened, species denoted as A21 and H22 showed the highest decolorization ability for Malachite green while fungus species denoted as A21 and B76 demonstrated the highest decolorizationfor Bromophenol Blue.

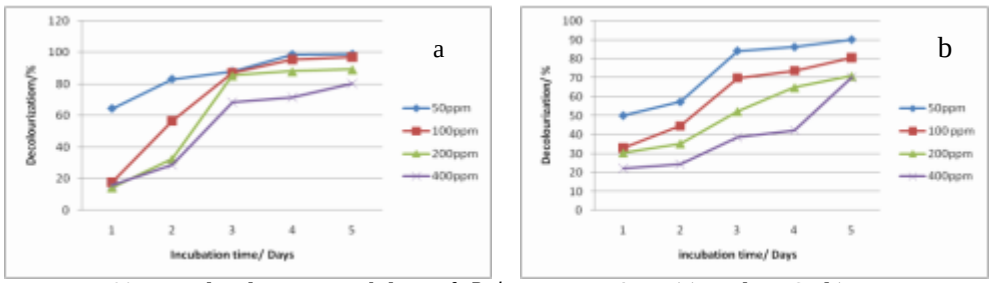

Figure 2. % Dye decolorization ability of *Polyporussp*. A21 (a) and B76 (b) against Bromophenol Blue

Polyporussp. A21 demonstrated over 80% dye decolorization with 50 ppm of Malachite green after five days of incubation (Fig.1a) while *Polyporussp*. H22 had over 80% dye decolorization with 50 and 100 ppm of Malachite green (Fig.1b). A decolorization ability of over 80% was achieved with both *Polyporussp*. A21 and B76 against Bromophenol Blue at 50 and 100 ppm after five days after incubation (Fig. 2.a and b). Highest decolorization ability was shown by species A21 at 50 ppm for both dye types tested. Dye decolorization ability decreases when dye concentration was increased to 200 and 400 ppm. Laccase enzyme activity showed a steady increase with time and reached the maximum level in day four and the dye decolorization ability also demonstrated a similar pattern. The highest laccase enzyme activity was shown by *Polyporussp*. A21 which also demonstrated the highest decolorization ability against both dyes tested. The results obtained in this study are in agreement with those of Ferreira et al. (2000) and Nyanghong at al. (2002) who also suggests that dye decolorization ability is closely related with the laccase enzyme activity. Decrease in colorization ability after day four could be related to less laccase enzyme secretion by aged fungal hyphae. By day four, the resources available in the culture medium could have been significantly depleted slowing the growth of the fungus thus reducing the release of laccase enzyme. Introducing fresh ME medium at day four could results in continuous dye decolorization ability even after day five. Identification of *Polyporus*species is a difficult task by observing the fruiting body and fungal mycelium alone. Adaptation of more sophisticated methods such as the use of molecular markers is sometimes necessary for accurate identification of fungal species. In this study, ten *Polyporus* species used in the initial screening were given code names and the selected species for quantitative dye decolorization assay, A21 and H76 were tentatively identified as *Polyporus sulphureus* and *Polyporus versicolor* respectively. Dye decolorization ability of *Polyporus sulphureus* was previously reported (Ferreira et al., 2000) yet, achieving over 80% dye decolorization ability with Malachite green and Bromophenol Blue was never obtained. Fungi species are prone for natural mutations and such mutant fungal strains could results in higher laccase enzyme activity and consequently high dye decolorization ability, as it was observed in this study.

Conclusions

This study identified three fungal species, A21, B22 and H76, as potential candidates for the treatment of textile dye effluents contain common dyes such as Malachite green and Bromophenol Blue. *Polyporussulphureus*and *Polyporusversicolor* demonstrated over 80%

dye decolorization ability when used at 50 and 100 ppm concentration. Dye decolorization ability of *Polyporus sulphureus*was previously reported yet. Achieving over 80% dye decolorization ability with Malachite green and Bromophenol Blue was never obtained. This study investigated the dye decolorization ability of selected *Polyporus* species under stimulated conditions using only one dye at a time. However, textile dye effluents from contain a mixture of various azo dyes and it would be an interesting future perspective of this study to investigate the performance of the selected fungal species under those actual conditions.

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