

Decolorization of textile dyes by edible white rot fungi

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SUMMARY

Properly disposing of wastewater-containing textile dyes has long been a problem for the textile industry. An innovative method, biodegradation, can degrade certain dyes more efficiently and in a more environmentally-friendly method than the commonly used physicochemical treatments. Fungus is one of the materials capable of biodegradation without requiring further processing. In this study, we tested the edible fungi, *Lentinula edodes*, *Pleurotus eryngii*, and *Pleurotus ostreatus*, to see if they can be used for a biodegradation of dyes. The textile dyes were applied into the suspension of these fungi for a biodegradation test, and the absorbance changes were recorded. After a reaction of one and a half hours, aniline blue dye and Congo red dye demonstrate the greatest degradation, reaching a biodegradation rate of 67.39–67.5% and 58.1–63.2%, respectively. Even the methylene blue dye that had the lowest degradation reached a biodegradation rate of 24.7–33.6%. These results show the fungi's capability to decolorize the dyes, presenting a potential method for biodegrading textile pollution and the possibility of using natural products to reduce polluted wastewater.

INTRODUCTION

To follow quick-changing fashion trends, consumers nowadays purchase and abandon clothes constantly. The rise of “fast fashion” promotes the growth of the economy, yet amplifies a problem that has existed for a long time. As one of the industries most responsible of pollution, the fashion industry faces the problem of treating wastewater that contains toxic or carcinogenic textile dyes (1-3). Without proper treatments, wastewater containing chemicals poses a great threat to the environment and can pollute drinking water or farming products (3). The study is meant to examine the ability of fungi to decontaminate wastewater.

Physicochemical treatments, such as coagulation-flocculation, advanced oxidation, and electrochemical techniques, are the most common method to decolorize wastewater in the industry because of their low price and low complexity (4). Since biological treatments are biodegradable, it has long been discussed and considered whether biomaterial could be used for the dye cleaning process (5). Of the two biological methods, biodegradation and biosorption, biodegradation is more efficient and could degrade dyes into harmless chemicals (6). Biodegradation involves two standard methods: Cell-based or enzyme-based biodegradation (7). Both methods clean the target materials

by degrading them with enzymes, but the enzymatic method is relatively weak, especially when functioning under extreme environments such as textile waste that contains chemical solutions with different pH levels. The cell-based method has a higher tolerance to extreme environments, since the cell can keep producing the enzyme in reaction condition (8). Also, the cell-based biodegradation method can be easy to remove after treatment. As a result, we tested cell-based degradation in this study.

White rot fungi species are the most widely used material for biodegradation (9). They produce multiple extracellular enzymes such as lignin peroxidases (LIPs), manganese dependent peroxidases (MNPs), and laccases, which allow them to biodegrade a wide range of dyes (9). Also, many white rot fungi are edible; therefore, the parts of fungi that cannot be sold for food could be used as biodegradation materials, decreasing waste from the fungi industry while treating textile waste (10, 11). We used three common edible white rot fungi in Taiwan, *Lentinula edodes*, *Pleurotus eryngii*, and *Pleurotus ostreatus* in this study; we expected to find a new workable fungus for biodegradation of textile dyes (12).

The textile industry uses acid, basic, direct, and azo dyes (13). In this study, we tested the biodegradation of Congo red (a direct dye), methylene blue (a basic dye), and aniline blue (an azo dye) because they are representative of compound structures in each group and are commonly used in the textile industry as well as other studies on biodegradation (Figure 1, 14-16). To summarize, this study aims to determine whether the fungi can decolorize the dyes and provide a new method to reuse fungi disposed by the fungi industry and biodegrade textile pollution.

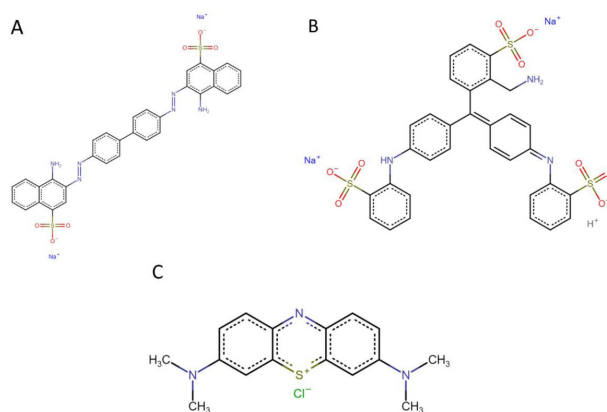


Figure 1: Chemical structures of dyes. (A) Congo red, (B) aniline blue, and (C) methylene blue, redrawn in Marvin JS according to the information from PubChem.

RESULTS

We mixed 0.5M dye and 1g of fungi in 10 ml of water and samples were taken every 45 minutes to determine degradation rates of each dye.

According to the experimental data, although *P. eryngii* showed a lower degrading effect on methylene blue dye during the early stage of the reaction, its final degradation rate was higher than the others (Figure 2A). A similar effect was seen with *L. edodes*' and Congo red dye (Figure 2C). Our results also demonstrated that the degradation of aniline blue dye (Figure 2B) occurred in the later stage of the reaction, whereas the degradation of Congo red dye (Figure 2C) occurred in the early stage of the reaction. Compared to the preceding results, the degradation of methylene blue (Figure 2A) showed a relatively equal distribution of degradation throughout the reaction.

The comparison between the biodegradation rate of each dye after 1.5 hours (Figure 3) showed that in the three fungi, methylene blue dye had the lowest degrading effect, reaching a biodegradation rate of 24.7–33.6% (Figure 3A). In the same time, the groups of aniline blue dye and Congo red dye demonstrated great degrading effect, reaching a biodegradation rate of 67.39–67.5% and 58.1–63.2% (Figure 3B,C), respectively. In addition, the degradation rate of aniline blue was slightly higher than the other two dyes. Surprisingly, although the degradation rates of dye on each fungus were not the same, there were not any significant differences.

DISCUSSION

Similar to other studies on fungi biodegradation, our experiments conducted on aniline blue showed a high biodegradation rate, demonstrating that *L. edodes*, *P. ostreatus*, and *P. eryngii* may be suitable for the biodegradation of azo dyes (17). The biodegradation rate of congo red was similar to that of aniline blue. The results of Congo red dye degradation suggested that these three fungi

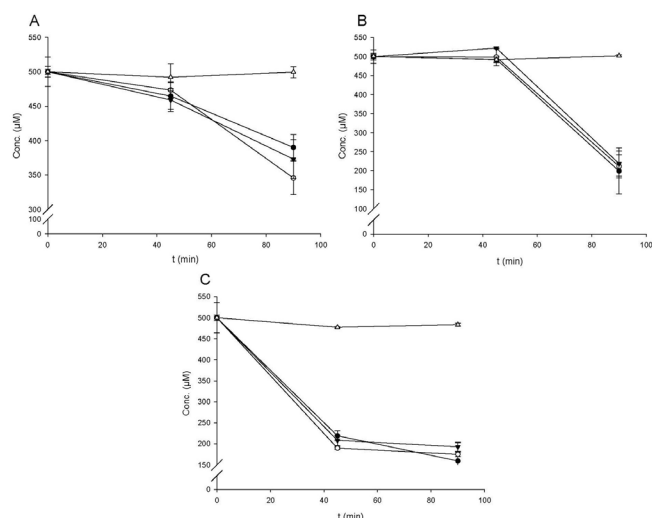


Figure 2: The change in dye concentration over time. Line graphs of the concentration of dyes, (A) methylene blue, (B) aniline blue, and (C) Congo red over time. The black dots represent *L. edodes*; the white dots represent *P. eryngii*; the black triangles represent *P. ostreatus*, and the white triangles represent control group. All the experiments were done in triplicate, and the dots represent the mean \pm SD of each condition.

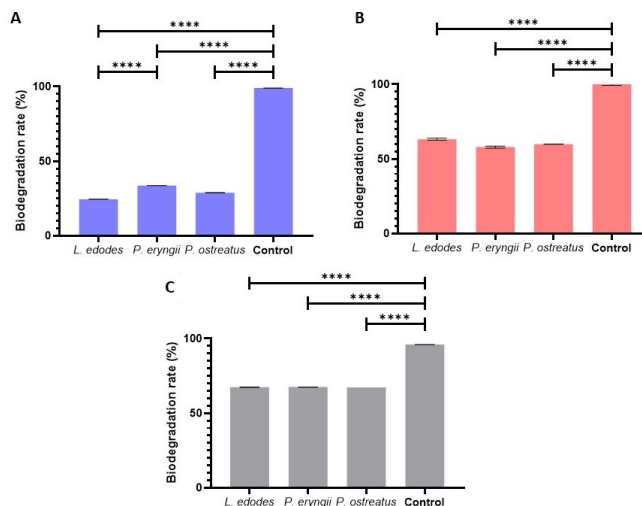


Figure 3: The biodegradation rate of each dye after 1.5 hours. (A) Methylene blue (blue bar). (B) Congo red (red bar). (C) Aniline blue (gray bar). All experiments were done in triplicate, and the different letters means statistically significantly different. All experiments were done in triplicate, and one-way ANOVA was used to analyze the statistical significance (**** $p < 0.0001$).

species are capable of degrading direct dyes. Comparison with previous studies suggest that biodegradation by fungi is more effective for Congo red than degradation performed with other methods, such as solar and UV lamps-based strategies (18).

We found that methylene blue biodegradation had a significantly lower effectiveness, which may be caused by the environment in which the reaction occurred. Other studies have suggested that methylene blue can reach a higher degradation rate in a more acidic environment (19). Furthermore, methylene blue dye may require more time to complete its biodegradation reaction in a less acidic condition. These assumptions can be verified further on in our studies.

Since the pH value of the reaction environment in which the biodegradation occurs may potentially affect the results, future research could explore how changes in acidity impact the degradation rates of each of the dyes and fungi species. The reaction time could be increased in further research as our data suggested that some dyes might require more time to be completely degraded. However, our data demonstrated the white rot fungi's ability to decolorize the dyes, representing a new, eco-friendly method for textile pollution biodegradation and the reuse of fungi disposed from the fungi industry.

MATERIALS AND METHODS

Fungi culturing

All equipment including the Potato Dextrose Broth (PDB) medium (NEOGEN, USA) and metallic tools were sterilized with an autoclave at 121°C for 20 minutes. The fungi (Wellcome, Taiwan), which bought from the local store, used in the study were cultured from the tissue of fungi acquired by transferring 0.1 mm² of mycelium tissue (the tissues used for culturing were taken from the middle of stalks to prevent contamination) onto the center of the PDB agar plate. The fungi were then cultured at 25°C in the dark for a week before subculturing into PDB medium for another week. The fresh

hyphae were harvested by centrifugation with 8000g at 4°C for 10 minutes and suspended in water to a final concentration of 0.1 g/ml.

UV-Visible Spectrophotometry

The highest absorption peaks of methylene blue (Sigma, USA), aniline blue (Scharlau, Spain), and Congo red (Sigma, USA) were determined by scanning their visible absorption spectrum at 664 nm, 600 nm, and 499 nm, respectively, using a UV-Visible spectrophotometer V-730 (JASCO, Japan). Five different concentrations of each dye were used to generate standard curves for calculation of detected concentrations based on Beer's law.

Biodegradation of dye

The reaction mix consisted of 0.5M dye and 1g of fungi in a total of 10ml water. Reactions were done at room temperature over 90 minutes. The concentration of the reacting dyes was measured three times, each time with an interval of 45 minutes. We calculated the biodegradation rate using the following formula:

$$\text{Biodegradation rate} = ((C_0 - C_t) / C_0) \times 100\%$$

C_0 was the dye concentration before treatment, and C_t was the dye concentration after treatment.

Statistical Analysis

The experiments were performed in triplicate. The statistical differences were calculated using one-way ANOVA from the analyze option of GraphPad Prism. All the figures of statistical analysis were performed by Sigma Plot and GraphPad Prism.

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