

BIOREMEDIATION OF TEXTILE WASTEWATER USING *Pleurotus pulmonarius*

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Abstract: The textile industry is a major polluter as it generates a high amount of wastewater on a daily basis. Conventional methods used to treat textile wastewater usually require a lot of energy and costs. Therefore, bioremediation using white rot fungi (*Pleurotus pulmonarius*) has been proposed because it produces lignin modifying enzymes, such as laccase, lignin peroxidase and manganese peroxidase, which breaks down pollutants in the wastewater. In this study, *Pleurotus pulmonarius* is used to treat dyes and selected heavy metals in textile wastewater obtained from a laboratory of a local university. The treatment used shake flask fermentation at a pH of 3, agitation speed of 120 rpm and temperature of 40°C to decolourise the dyes and reduce the heavy metal content. *P. pulmonarius* showed promising results with a maximum of 59.45% of dyes being decolourised after 144 hours. Furthermore, the level of heavy metals, as determined through Inductive Coupled Plasma Optical Emission Spectrometry, is reduced to 34.54%, 76.82%, 38.17% and 41.94% for copper, iron, manganese and zinc, respectively, after 144 hours of incubation. However, the biochemical oxygen demand of the wastewater increased with treatment, and the fungi cells are not viable anymore after 72 hours of incubation. With further optimisation, *P. pulmonarius* seems to be a promising bioremediating agent for treatment of textile wastewater.

Keywords: *Pleurotus pulmonarius*, white rot fungi, bioremediation, wastewater, dye, heavy metals.

Introduction

Water pollution is a growing concern as it is becoming a greater threat to all life forms. As defined by Schweitzer and Noblet (2018), water pollution is the impairment of a given water body with the presence of chemical, physical or biological components. In Malaysia, the 2019 incident in Sungai Kim Kim, Johor, Malaysia, had caused thousands of people, mostly schoolchildren, to fall ill and schools were closed for weeks as toxic fumes were emitted from chemicals dumped into the river. Sporadic cases in rivers in the state of Selangor between 2020 and 2021 had caused water supply disruptions to millions of households in the Klang Valley amid the COVID-19 pandemic.

The textile industry has been identified as a significant contributor of water pollution as it is one of the most chemical-intensive industries

that consume and discharge large volumes of water on a daily basis. The wet processes of the industry, such as singeing, desizing, kiering and bleaching, require large quantities of water (Patel & Vashi, 2015). In fact, about 200 L of water is used per kg of fabric processed daily (Kant, 2012; Khatri *et al.*, 2015). Generally, the textile wastewater is unstable in various parameters, such as biochemical oxygen demand (BOD), pH level, colour and salinity (Chequer *et al.*, 2013).

Dye has been identified as the main pollutant, which is hard to degrade and persistently found in the environment (Couto, 2009). In fact, dyes are believed to be the most difficult component to treat, and conventional methods have proven to be ineffective (Doble & Kumar, 2005; Couto, 2009). Furthermore, other problems, such as high cost and energy level consumed, need for specialised equipment, and production of secondary contaminants make existing textile

wastewater treatment methods less efficient and unfavourable (Dos Santos *et al.*, 2007; Adegoke & Bello, 2015; Katheresan *et al.*, 2018).

Bioremediation is a promising alternative to overcome the problems posed by other textile wastewater treatments. By using living organisms to treat pollutants, bioremediation is efficient, yet low in cost and is compatible with the environment (Ali *et al.*, 2009). The white-rot fungi have been commonly studied due to their non-specific action of lignin-modifying enzymes that help break down pollutants. These enzymes, such as laccase, lignin peroxidase and manganese peroxidase, may effectively biodegrade textile dyes through the generation of free-radicals (Mir-Tutusaus *et al.*, 2018). The action of these enzymes is further enhanced by the structural similarity of the dyes to lignin (Wesenberg *et al.*, 2003). Hence, this group of fungi is considered a promising alternative in treating textile wastewater.

This study tries to determine the efficiency of the white-rot fungus *Pleurotus pulmonarius* in treating textile wastewater. The ability of the fungi to treat wastewater is characterised by the total percentage of decolourised dye, the biochemical oxygen demand (BOD) level before and after treatment, the reduction of several heavy metal content, and the viability of fungal cells throughout incubation. To the best of our knowledge, this study is a novel attempt in using *P. pulmonarius* to treat textile wastewater.

Materials and Methods

Chemicals

The Oxoid® potato dextrose agar (PDA) and potato dextrose broth (PDB) (Thermo Fisher Scientific, Hampshire, UK) were used in the cultivation of *P. pulmonarius*. Sodium hydroxide (Sigma-Aldrich, Seelze, Germany) was used to set the pH value of the textile wastewater.

Fungal Cultures

P. pulmonarius was purchased from the Forest Research Institute Malaysia (FRIM) in Kuala

Lumpur, Malaysia. It is a well-studied white-rot fungus that was able to produce ligninolytic enzymes. The fungi culture was maintained on PDA and by spore suspensions at 4°C until further use.

Textile Wastewater

The textile wastewater was obtained from a textile technology laboratory at the Faculty of Applied Sciences, Universiti Teknologi Mara (UiTM), Shah Alam, Selangor, Malaysia, and kept in a high-density opaque jerry can. Reactive and acid dyes were the main chemicals studied in the UiTM textile laboratory. Upon collection, the pH value, absorbance and temperature of the water were recorded. The textile wastewater was then filtered twice through 0.45 µm filtration membranes to remove debris (Hamed *et al.*, 2014). It was immediately added to *P. pulmonarius* cultures after collection to avoid potential decolourisation by external factors or chemical instability.

Decolourisation of Dyes

The textile wastewater was adjusted to pH 3 using sterile 1 M sodium hydroxide solution prior to seeding. A total of 150 ml of the textile wastewater was seeded with 20% (v/v) of *P. pulmonarius* cultures grown in PDB in a 250-ml Erlenmeyer flask. A negative control flask was prepared without inoculation. The flasks were put onto an incubator shaker at a temperature of 40°C and agitation speed of 120 rpm. The absorbance of the wastewater culture was recorded every 24 hours at a wavelength of 495 nm for a total of 144 hours. The wavelength was the maximum used to determine absorbance for textile wastewater as determined in a preliminary study. The 144-hour incubation period was chosen as the death phase of *P. pulmonarius* culture would begin around that time as determined in a growth profile (data not shown). The percentage dye decolourisation (PDD) is calculated using Equation 1 (1) as stated by Rani *et al.* (2014).

$$\text{PDD}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance inoculated}}{\text{Absorbance of control}} \times 100 \quad (1)$$

Determination of Biochemical Oxygen Demand

The BOD levels of the textile wastewater prior to and after incubation with *P. pulmonarius* were measured according to the Five-Day Biochemical Oxygen Demand method detailed by Delzer and McKenzie (2003). The wastewater samples were prepared by adjusting the pH to 7 by addition of 1 M sodium hydroxide, and

then serially diluting them 100 times (10^{-2}) using BOD dilution water containing nutrient salts. The diluted textile wastewater was then used to fill a 300-ml BOD bottle and the initial dissolved oxygen concentration was measured using a dissolved oxygen (DO) meter before incubation at 20°C. The final dissolved oxygen levels were measured using the DO meter after five days, and the BOD level of the wastewater was calculated using Equation 2 (2).

$$\text{BOD Level} = \frac{\text{Final dissolved oxygen concentration} - \text{Initial dissolved oxygen concentration}}{\text{Sample Dilution}} \quad (2)$$

Determination of Heavy Metal Concentration

The textile wastewater culture was collected prior and after 144 hours of incubation with *P. pulmonarius*. It was filtered to get rid of the fungal biomass. The Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) model Optima 7300 DV (Perkin Elmer, Waltham, MA, USA) was calibrated with 10 ppm standards of copper, iron, manganese and zinc, and sterile deionized water serving as a blank. After calibration, the heavy metal concentration of the samples was immediately measured.

Viability of Cells

Everyday within the 144-hour incubation period, 0.2 ml of the inoculated textile wastewater was plated onto fresh PDA and incubated for five days at 28°C. The fungal growth was recorded daily.

Statistical Analysis

The data for the percentage of dyes decolourised was analysed using IBM SPSS Statistics Version 25 (IBM Corp, Armonk NY, USA). A single-sample *T*-test was used to determine if there was significant difference between the percentage of dye discoloration over the incubation period. A value of $p < 0.05$ was considered statistically significant.

Results and Discussion

Textile Wastewater Collection

The textile wastewater collected was purple colour, with a layer of brown oil on top. It had a very strong paint odour. Upon collection, the pH value and temperature were recorded at 0.98 and 25°C, respectively. Textile wastewater typically had an average pH of 6 to 10 (Upadhye and Joshi, 2012; Kehinde and Aziz, 2014). However, the very-low pH value in this study's wastewater might be due to the difference in chemicals and treatments used while dyeing and treating textiles.

Decolourisation of Textile Wastewater

After adjusting the pH value of the textile wastewater to 3, the wastewater was seeded with *P. pulmonarius* and incubated. The experiment was conducted in triplicates. The fungal cells managed to decolourise some of the textile wastewater. The bioremediation profile is shown in Figure 1.

P. pulmonarius achieved high levels of dye decolourisation, but it did not achieve complete decolourisation. Immediately after seeding, 12.26% of the dyes in the textile wastewater were decolourised. The percentage exponentially increased until 72 hours of incubation, whereby 44.23%, 47.36% and 55.71% of dyes in the

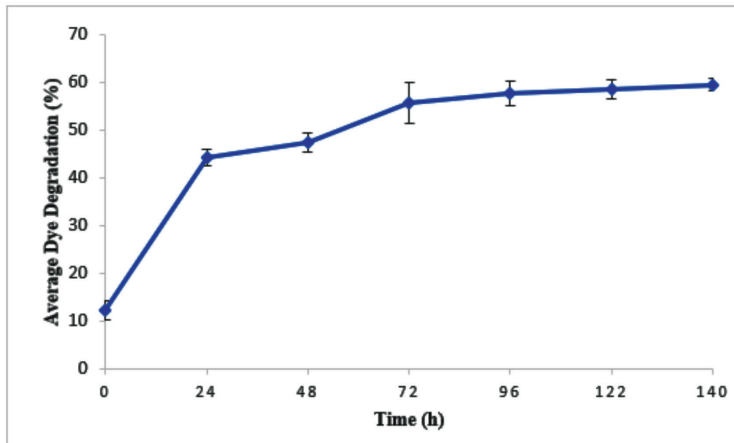


Figure 1: Profile of bioremediation of textile wastewater by *P. pulmonarius* at 40°C, agitation rate of 120 rpm and pH 3 ($P < 0.05$)

wastewater were decolourised at hours 24, 48 and 72, respectively. However, after 72 hours, decolourisation began to slow down and almost became constant at 57.68%, 58.42% and 59.45% at 96, 122 and 144 hours, respectively. The *t*-test showed that each reading per day was significantly different ($p < 0.05$).

The pH value, temperature and agitation rate were chosen based on a preliminary study that observed the optimal condition for *P. pulmonarius* to degrade Reactive Yellow 125 dye (data not shown). Despite existing studies such as Souza *et al.* (2002) stating that the laccase of *P. pulmonarius* was unstable at acidic pH values, pH 3 was found to be the best condition for the white-rot fungi *Pleurotus eryngii* F032 to thrive in the study by Hadibarata *et al.* (2013). Even when using the laccase of the ascomycete endophyte *Paraconiothyrium variabile*, Mirzadeh *et al.* (2014) found pH 3 to be the best pH value for dye decolourisation. A study by Bazrafshan *et al.* (2012) determined that pH 3 was also the best condition for adsorption of dyes onto their single-walled carbon nanotubes due to the protonation of electron rich regions of the surface, allowing better uptake of negatively charged dyes.

Shake flask fermentation was implemented at an agitation speed of 120 rpm as it was proven that agitated cultures could achieve better

decolourisation than static ones as demonstrated in the study by Hadibarata *et al.* (2012). Agitation allowed for the equal distribution of substrate mass, heat transfer, nutrient availability and dissolved oxygen levels (Venugopal *et al.*, 2007; Ibrahim *et al.*, 2015). However, an agitation that was too rough might harm the cell cultures through collision and shear forces, as pointed out by Ibrahim *et al.* (2015). The implementation of 120 rpm was reportedly the best speed for dye decolourisation using several white-rot fungi species in Adnan *et al.* (2016) and Hadibarata *et al.* (2013).

In this study, 40°C was chosen as the incubation temperature, which was outside the range of usual temperatures used to cultivate *P. pulmonarius*, which was between 5°C to 35°C. However, this higher temperature had been reported as optimal for free laccase activity by Mazlan and Hanifah (2017). Moreover, Hadibarata *et al.* (2013) also found that 40°C was the best temperature for dye decolourisation in their white-rot fungus culture. This can be credited to the solubility of laccases, which increased with high temperatures (Saratale *et al.*, 2009).

Biochemical Oxygen Demand

The changes in BOD level of the textile wastewater before and after treatment using *P.*

pulmonarius were determined using the five-day BOD test. The results are illustrated in Figure 2.

Interestingly, the BOD levels of both negative control and the inoculated flasks with *P. pulmonarius* had increased after incubation. The level of the negative control increased from 708 mg/L to 729 mg/L, while the level of the samples treated with *P. pulmonarius* increased from 709.33 mg/L to 735.67 mg/L. The high levels of BOD were not unusual, as such typical wastewater might have BOD levels ranging from 80 to 6000 mg/L (Ananthashankar, 2013).

The increase in BOD levels for both samples might be due to the incubation conditions. The United States Environmental Protection Agency stated in 2012 that the rate of oxygen consumption in water bodies was affected by temperature, pH, the presence of microorganisms, and organic and inorganic matter in the water. Since the temperature used in this study was higher than room temperature, it might have caused the increase in BOD level as it supported the multiplication and metabolism of microorganisms, as well as hydrolysis of organic compounds, leading to higher availability of organic matter (Metcalf & Eddy, 2003). This, in turn, would increase the rate of oxygen depletion in the water.

In some bioremediation studies, BOD levels were reduced by the inoculum. In the study by Haroun and Idris (2009), when an anaerobic fluidised bed reactor was implemented in the bioremediation of textile wastewater, 95% of the BOD level could be successfully reduced. However, it should be noted that this study was done during a 70-day period using the bioreactor. In another study, Dhaouefi *et al.* (2018) were able to reduce a large portion of the BOD level by implementing an anoxic-aerobic photobioreactor containing microalgae to treat textile wastewater. Both studies used complex bioreactor systems instead of the simple shake flask fermentation system. Hence, further studies using similar systems would be recommended to truly investigate the efficiency of *P. pulmonarius* in reducing BOD during bioremediation of textile wastewater.

Reduction of Heavy Metal Concentration

Figure 3 shows the percentage of average total reduction of metal after the treatment as measured using ICP-OES.

Not much difference was observed between the negative control and the samples treated with *P. pulmonarius*. Only iron showed a notable difference, where *P. pulmonarius* reduced

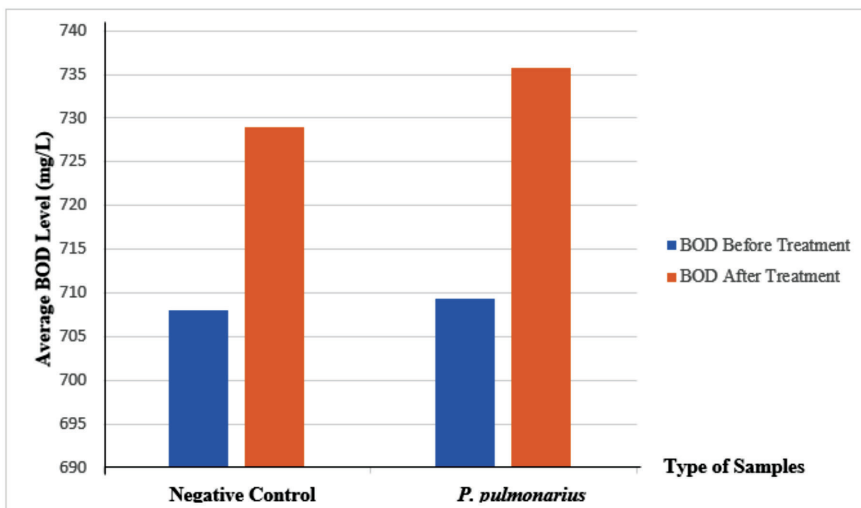


Figure 2: Average BOD levels of textile wastewater before and after treatment

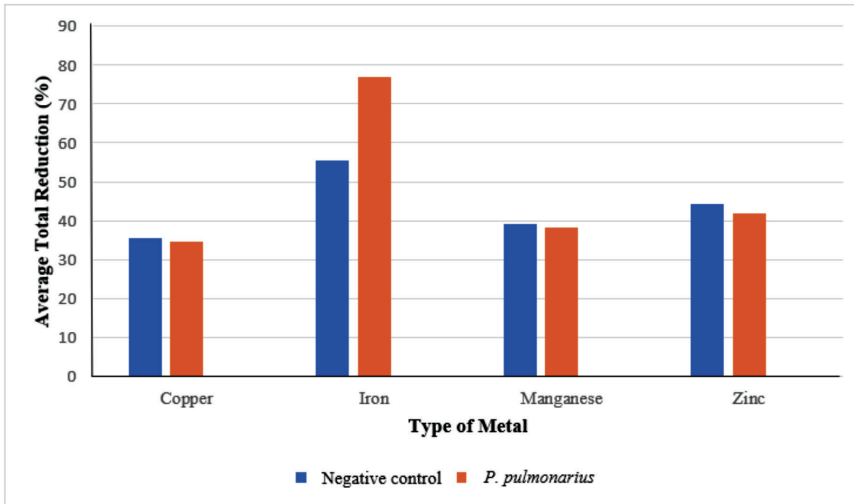


Figure 3: The average total reduction of copper, iron, manganese and zinc after incubation at 40°C, 120 rpm and pH 3 for 144 hours

76.82% of the content in the wastewater compared with 55.45% in the negative control, however, an independent samples *t*-test found that the difference in reduction is not statistically significant ($p > 0.05$). Similar patterns were observed for copper, manganese and zinc, where the negative control had reduced slightly more than *P. pulmonarius*. The reduction in heavy metal levels in the negative control might be attributed to the addition of NaOH solution when adjusting the solution pH at the start of the experiment. The conditions might have also allowed interaction between pre-existing chemicals, such as surfactant agents in the wastewater.

The heavy metals copper, iron, manganese and zinc were of interest because of their toxic effects towards the water system. For example, copper had been found to promote ocean acidification and global warming when present in the water system, hence disrupting marine life such as microalgae (Leal *et al.*, 2018). Iron, on the other hand, might disrupt the supply of drinking water by discolouration and leaving a bad taste (Behera *et al.*, 2012). Manganese had been associated with neurotoxicity in children that disrupted their psychomotor development

(Röllin, 2011). In fact, in a study by Fu *et al.* (2016), copper and zinc were found to be more detrimental towards aquatic life in a large urban lake in Eastern China compared to other heavy metals. Some of these metals were also cations of dyes, which allowed the dye to persist in the environment and present a cumulative effect in the food chain, eventually affecting the health of humans (Vargas *et al.*, 2009; Copaciu *et al.*, 2013).

The *Pleurotus* species could remove heavy metals in wastewater by means of biosorption. However, the degree of tolerance differed for each species and type of heavy metal (Kapahi & Sachdeva, 2017). The heavy metals would be absorbed and accumulated in the fruiting bodies of the fungi (Adongbede & Okhuoya, 2011). The removal of heavy metals may also take place by chemisorption and ion-exchange (Kapahi & Sachdeva, 2017). Iron may have been more successfully reduced by *P. pulmonarius* compared to other metals due to the action of its exopolysaccharides. This was supported by Ogidi *et al.* (2020), which found that the exopolysaccharides of *P. pulmonarius* grown in agro-waste could scavenge up to 84.70% of free ferro ions (Fe^{2+}).

Cell Viability

The viability of the fungal cells throughout the incubation period was determined by plating 0.2 ml of the inoculated textile wastewater onto fresh PDA daily and incubated at 28°C. The plates were then observed daily for seven days for colony growth. Table 1 shows the results of the experiment.

No growth was detected in the negative control throughout the incubation period, signifying that no contamination had occurred in the wastewater and control during the experiment. Furthermore, it also indicated that the decolourisation and heavy metal reduction occurred not by contamination, but rather, instability of the wastewater.

P. pulmonarius was not viable throughout the incubation period, as most of the cultures began dying after the 72nd hour. This could be due to the sensitivity of the fungi towards the accumulation of heavy metal concentrations and acidic end-products of fermentation. The study by Palmans *et al.* (2015) found that *Pleurotus* species were more sensitive to high levels of heavy metals.

The non-viability of the cells also corresponded to the plateauing of the decolourisation percentage. As fewer fungal cells were viable, less extracellular enzymes would be produced, hence less decolourisation occurring. When the cells were non-viable, decolourisation occurred only through biosorption. The non-viable cells were also regarded as organic matter which in turn, would increase the BOD level of the wastewater.

Conclusion

P. pulmonarius had the potential to treat textile wastewater under an optimised condition. During the 144 hours of incubation, a total of 59.45% of dyes in the textile wastewater in this study was decolourised, albeit with an increase in BOD level. Despite that, iron levels were also successfully reduced. However, the cells were no longer viable after the 72nd hour of incubation. With further optimisation, *P. pulmonarius* might be a promising alternative in the treatment of textile wastewater as it was able to bioremediate the wastewater to a certain extent. More factors that maintained the viability of *P. pulmonarius* should be studied, such as the effects of carbon and nitrogen sources. Moreover, it was recommended that the metabolic end-products of the bioremediation process be identified and removed to avoid the accumulation of toxic end-products for *P. pulmonarius*.

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Table 1: Viability test of samples during incubation

| Sample | Time (hours) | | | | | | |
|---------------------------|--------------|----|----|----|----|-----|-----|
| | 0 | 24 | 48 | 72 | 96 | 120 | 144 |
| Negative Control | - | - | - | - | - | - | - |
| <i>P. pulmonarius</i> (1) | + | + | + | - | - | - | - |
| <i>P. pulmonarius</i> (2) | + | + | + | + | - | - | - |
| <i>P. pulmonarius</i> (3) | + | + | + | + | - | - | - |

(+): Growth; (-): No growth

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