

Biosorption of Synthetic Dye by Macrofungi

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ABSTRACT: This research project aimed to provide an environmentally friendly method for the decolorization and biosorption of synthetic dye by utilizing fungi as biosorbents. The study was carried out by first growing the fungi in solid medium and then using the fungi as biosorbent to absorb dye in aqueous solution. In the first stage, screening experiments were carried out among 5 different types of fungi, and *Pleurotus ostreatus* was determined to have the highest growth rate. The *Pleurotus ostreatus* was recultivated with Remazol Brilliant Blue R dye to determine its dye removal ability. *Pleurotus ostreatus* sp. exhibited vigorous dye decolorization in agar medium within 2 days. By carrying out batch analysis, 4 parameters were examined, which were the effect of pH, surfactant concentration (Tween 80), salinity concentration and dosage of biosorbent. The results showed that the maximum dye decolorization by *Pleurotus ostreatus* can be achieved through establishing an acidic condition of pH 2, addition of 0.1mL of Tween 80, 0mg/l of sodium chloride concentration, and dosage of 8 plugs. Lastly, the experimental data was found to fit the Jovanovic Isotherm the most. In conclusion, *Pleurotus ostreatus* is capable of decolorizing and adsorbing dye particles in the dye aqueous solution.

KEYWORDS: Biosorption; Remazol Brilliant Blue R; biosorbent; decolorization; *Pleurotus ostreatus*

1. Introduction

Among the industries, the water pollution caused by the textile industry is one of the public's main concerns as the dye effluent released from the textile industry contains a high

concentration of suspended solids, Chemical Oxygen Demand (COD), and heavy metals such as Cadmium, Zinc, Nickel, and Lead. Previous studies showed that 20% of global water pollution issues were caused by the textile industry, and it is estimated that more than 15% of dyes are released in leftover liquors from incomplete exhaustion of dyeing processes. The discharge of effluent from commercial and industrial sectors, as well as untreated sewage such as organochlorine, organophosphate, heavy metals, pharmaceuticals, herbicides, and textile dye, are among the compounds released into freshwater supplies that are harmful to humans, animals, plants, and the ecosystem as a whole. Those dye wastewater treatment systems that are currently available could only be able to reduce the dye lost through effluent by 45% [1-3].

There are a variety of chemical, physical, and biological procedures available for removing dye from aqueous solutions. Various approaches, such as biosorption, chemical precipitation, electrochemical treatment, and reverse osmosis, are being researched for the removal of hazardous metals from aqueous media. Biosorption has been the subject of much research for the removal of harmful metals from aqueous solutions among existing treatment methods. Biosorption is the sequestration of organic and inorganic species, such as metals, dyes, and odor-causing compounds, by utilising living or decomposing biomass or its derivatives. This biomass may consist of bacteria, fungi, algae, sludge from biological wastewater treatment facilities, fermentation industry byproducts, or seaweed [4,5]. The adsorbents in this procedure are biological materials, and the primary removal method is sorption. For the removal of heavy metals and other contaminants from wastewater, biosorption has been continually investigated since the 1980s, and thus it could be a potential alternative to replace or complement existing dye-bearing wastewater treatment methods. When compared to other available technologies, including precipitation, ion exchange, reverse osmosis, and adsorption, biosorption provides comparable performance at a cheap cost [6-11].

Macrofungus, among the frequently accessible sorbents, possesses the qualities of an ideal biosorbent. It is readily available and inexpensive everywhere, particularly in regions with hot and humid climates. A biosorbent derived from macrofungi is chemically stable in the majority of alkaline and acidic environments [12,13]. Some prokaryotic and eukaryotic microorganisms are capable of producing extracellular polymeric substances such as polysaccharides, glucoprotein, lipopolysaccharide, soluble peptides, etc. It is possible that the relative contributions of lignin peroxidase, manganese peroxidase, and laccase to the decolorization of dyes vary between fungi. A biosorption mechanism may also play an essential part in the decolorization of dyes by live fungi, in addition to biodegradation. Adsorption, which involves physico-chemical interactions such as adsorption, deposition, and ion-exchange, is the mechanism for dead cells. Additionally, it has excellent mechanical qualities against abrasion. The complexity of the fungal cell wall structure indicates that there are many ways for the dye to be absorbed by the fungi. The biosorption mechanism of fungi can be divided into 2 forms, which are active and passive metal uptake. Active uptake of metal ions depends on the metabolism of fungi in which the process requires energy to occur. Passive metal ion uptake is not dependent on fungi metabolism because biosorption processes do not require energy [14,15]. The aim of this research project was to identify the fungi specie with the highest adsorption of dye. Equilibrium and kinetic studies were used to determine the adsorption mechanism studies.

2. Materials and Methods

2.1. Biosorbents

The biosorbents used in the study were prepared from five dead macro fungi (mushrooms), *Hypsizygus tessellatus* (buna shimeji mushroom), *Lentinula edodes* (shiitake mushroom), *Pleurotus eryngii* (king oyster mushroom), *Pleurotus ostreatus* (oyster mushroom), and *Flammulina velutipes* (Japanese enoki). The macro fungi were hand-harvested, rinsed with water, cut into little pieces, and dehydrated at 40°C for 48 hours. An electric ball mill was used to grind the dry adsorbents. The sorbents were sieved using a 600 µm mesh to exclude larger particles. The sieved adsorbents were kept in an oven at 40°C for 24 hours before being put in a desiccator without any other chemical or physical treatment.

2.2. Adsorbates

An anthraquinone type dye, remazol brilliant blue r (RBBR) was selected as the representative textile dyes. RBBR is one of the indicators to check the production of ligninolytic enzyme by microbes. The ability of microbes to show the positive reaction in this indicator corresponds with the lignin decomposition, and it is regarded as prognostic of its main role in the degradation of xenobiotic compounds such as pesticide, halogenatic compounds, and polycyclic aromatic hydrocarbons [5]. Aqueous dye solutions of 100 and 200 mg/L were prepared in extraction water. The extraction water was pre-pared using ultra-pure (Millipore) water and 0.5, 0.3 and 0.2 mmol/l NaHCO₃, CaCl₂.2H₂O and MgSO₄.7H₂O, respectively. The pH of extraction water was adjusted to 7.5 ± 0.2.

2.3. Screening and selection of fungi

The five fungi were screened using malt yeast extract agar on a 9-mm Petri dish. After autoclaving, 100 mg/L of chloramphenicol or benomyl was added to the agar media to prevent bacterial growth as well as RBBR. The favorable responses were indicated by the growth of the chosen strain on agar plates. As a negative control, one flask devoid of fungi is used to determine any volatilization of the chemical indicator. The culture was incubated at 28° C for at least seven days in the darkroom. Each experiment was performed three times.

2.4. Analytical method

On the magnetic stirrer, a beaker glass containing 1l of distilled water and 5 g of malt yeast extract is placed to ensure that the mixture is thoroughly combined. Using a tensette pipet, 20 mL of the solution is transferred to the 100-mL conical flask. The conical flask is wrapped in aluminum foil and autoclaved for 1 h at 125°C. This procedure was required for the sterilization of glassware and liquid media. The conical flask was then filled with a 20g/L glucose solution that had been previously autoclaved. It is necessary to add glucose solution after autoclaving because glucose is capable of releasing a toxin when autoclaved with other nutrients [16,17]. The dye concentration in the conical flask is at 0.025g/L. It is crucial to add the dye after autoclaving the liquid medium, as the high temperature of autoclaving disrupted the structure of the dye. The sample solution was filtered with advantec filter paper, and the dye removal was determined by calculating the decrease in UV absorbance at 590 nm (RBBR) (Perkin Elmer) [12]. The dye removal (%) and adsorption capacity q_e (mg/g), was calculated by:

$$\text{Colour removal (\%)} = \frac{C_i - C_f}{C_i} \times 100$$

$$\text{Adsorption capacity (mg/g)} = \frac{C_i - C_f}{M} \times V$$

where C_i is initial concentration of dye solution (mg/L), C_f is final concentration of dye solution after adsorption (mg/L), M is the mass of adsorbent (gm) and V is the volume of dye solution (L).

There are several parameters to be tested in the research study which are effect of pH, effect of salinity, surfactant concentration and dosage of adsorbent. The variation in parameters are summarize in the Table 1.

Table 1. Summarizes for all experiments of batch adsorption.

Parameters	Materials	Range
pH	HCl or NaOH	2, 4, 7, 10
Salinity (mg/L)	NaCl	0, 50, 100, 150
Surfactant (mL)	Tween 80	0.1, 0.5, 1, 1.5
Dosage of fungi	5mm of grown fungi	2, 4, 6, 8

Using adsorption isotherm models, the efficiency of the interaction between biosorbents and dye solution can be evaluated. In adsorption isotherm calculations, surface properties, adsorption capacity, and the efficiency of the adsorption system are all considered. This experiment involves four adsorption isotherms, namely the Langmuir, Freundlich, Harkin-Jura, and Jovanovic isotherms (Table 2).

Table 2. Adsorption isotherm of the study.

Isotherm	Equation
Langmuir	$\frac{C_e}{q_e} = \frac{1}{q_m K_L} + \frac{C_e}{q_m}$
Freundlich	$\log q_e = \log K_F + \frac{1}{n} \log C_e$
Harkin-Jura	$\frac{1}{q_e^2} = \frac{B}{A} - \left(\frac{1}{A}\right) \log C_e$
Jovanovic	$\ln q_e = \ln q_{max} - K_J C_e$

where C_e is concentration of adsorbate at equilibrium (mg/g), q_e is amount of adsorbate at equilibrium (mg/g), q_m is equal to q_e and K_L is Langmuir constant related to adsorption capacity (L/mg), K_F is adsorption capacity (mg/g), B represents the Harkin-Jura constant from the graph plotted of $1/q_e^2$ versus $\log C_e$, A represents the Harkin-Jura constant from the graph plotted of $1/q_e^2$ versus $\log C_e$, q_{max} represents the maximum amount of adsorbate in equilibrium, K_J represents the Jovanovic constant.

3. Results and Discussion

3.1. Screening of fungi

Pleurotus ostreatus grew at the fastest rate (86.17%), followed by *Pleurotus eryngii* (77.97%). Due to the fact that both species belong to the genus *Pleurotus*, which is recognized by its gilled structure, the growth rates of both species were relatively comparable (Table 3). The optimal growing temperature for *Pleurotus* is 27° C at room temperature. Previous research on the

influence of temperature on the mycelium and fruiting development of *Pleurotus ostreatus* [18] shown that the mycelium grew significantly at 27 °C during incubation. In addition, yeast extract is essential to the growth of mycelium in fungi. Yeast extract is frequently added to growing medium since it is a rich source of nutrients. Yeast extract can stimulate the growth of fungi by providing them with vitamins, minerals, and nucleic acid [19,20].

Table 3. Growth of the fungi

Fungi	Growth rate (%)
<i>Hypsizygus tessellatus</i>	51.39
<i>Lentinula edodes</i>	72.92
<i>Pleurotus eryngii</i>	77.97
<i>Pleurotus ostreatus</i>	86.14
<i>Flammulina velutipes</i>	14..72

3.2. Effect of pH

The effects of some parameters on dye removal and adsorption capacity of RBBR by *Pleurotus ostreatus* are shown in Table 4. The elimination of RBBR by *Pleurotus ostreatus* was determined to be 88.23%, 72.44 %, 71.18 %, and 30.73% for pH values of 2, 4, 7, and 10 correspondingly. pH is a fundamental parameter that has a major impact on the decolorization of dyes by macro fungi. Acidic circumstances appeared to be more advantageous for decolorization of the dye Remazol Brilliant Blue R by *Pleurotus ostreatus*; nonetheless, high decolorization was accomplished even at a neutral pH of 7. The fungus exhibited ruderal characteristics, as demonstrated by its rapid growth in liquid medium after only 15 days. The vast majority of fungi thrive in pH 3 to pH 6 environments. In this experiment, however, *Pleurotus ostreatus* was able to survive at a pH of 2. Existing research indicates that *Pleurotus ostreatus* reacts differently in terms of its ability to adjust to their environment's pH. The optimal pH range for laccase and lignin peroxidase activity was between 3 and 7. Since acidic conditions are more hospitable to fungi, their biomass production and enzyme secretion, which aid in the biosorption of dye, are increased.

Table 1. Effect of some parameters on dye removal and adsorption capacity of RBBR by *Pleurotus ostreatus*.

Paramaters	Dye Removal Rate (%)	Adsorption Capacity (mg/g)
pH		
2	88.23	0.303825
4	72.44	0.249466
7	71.18	0.245095
10	30.73	0.105803
Dossage		
2	70.23	0.725509
4	74.27	0.383643
6	81.15	0.279452
8	85.48	0.220759
Salinity		
0	89.89	0.309552
50	88.15	0.303531
100	41.59	0.143223
150	24.44	0.84157
Surfactant		
0.1	83.36	0.287041
0.5	67.22	0.231484
1.0	63.02	0.217004
1.5	53.93	0.185705

In terms of electrostatic interaction, the positively charged surface of the biosorbent attracts the negatively charged anions of the dye. Therefore, the dye anions bond to the biosorbent's cell wall. This mutual attraction results in the decolorization of the dye. Low pH

can enhance the availability of H^+ ions for the binding of negatively charged organic compounds to the cell wall of fungus. Previous research demonstrated that the isolated species of *Achaetomium Strumarium* had the highest dye decolorization rate of 98.99% on Acid Red 88 dye at pH 4. A comparable decolorization rate, 76.77%, was observed at a pH level of 7. *Trametes villosa* sp. achieved the maximum decolorization rate of 94.03% at pH 5. However, at a pH of 9, the rate of decolorization decreased to 53.11 percent. This showed that alkaline conditions had a big effect on *Trametes villosa* sp, since the dye lost its colour gradually as pH went up [21,22].

3.3. Effect of biosorbent dosage

For dosages of 2, 4, 6, and 8 plugs, the removal of RBBR by *Pleurotus ostreatus* sp was calculated to be 70.23%, 74.27%, 81.15%, and 85.48%, respectively. Conclusion: the number of dosages is directly proportional to the rate of dye decolorization. The increase in decolorization rate from 2 to 8 plugs could be attributed to the increasing surface area of the biosorbent [23,24], which is responsible for the binding of metal ions and organic dye molecules to the biosorbent. Increases in surface area essentially led to the availability of more active binding sites for the attachment of dye anions to the biosorbent. Previous research demonstrated the correlation between the surface area of biosorbent and its absorption ability, with fungus having the highest decolorization rate. It was attributed to the enhancement of the absorption process [23,25].

3.4. Effect of surfactant concentration

With the addition of Tween 80 at concentrations of 0.1 mL, 0.5 mL, 1.0 mL, and 1.5 mL, the dye decolorization rate was determined to be 83.36 percent, 67.22 percent, 63.02 percent, and 53.93 percent, respectively. It might be owing to the potential of Tween 80 molecules to be absorbed by solid surfaces, or it could be due to the sorption ability of Tween 80 molecules. In some instances, it has been observed that surfactants slow the biodegradation of organic molecules. Similar results were obtained in prior work utilising *Trichoderma lixii* F21 to decolorize alizarin red s and quinizarine green ss [19]. Inversely related to the concentration of Tween 80 was the decolorization of mordant orange-1. However, it was proportionate to the rate of biomass production. Nor et al. also observed that *Trichoderma harzianum* sp. achieved the maximum decolorization of Cresol Red (88%) at a low surfactant dosage of 0.1 mL of Tween 80 [26].

3.5. Effect of salinity

With the addition of sodium chloride at concentrations of 0mg/L, 50mg/L, 100mg/L, and 150mg/L, the dye decolorization rate was determined to be 89.89%, 88.15 %, 41.59%, and 24.44%, respectively. Salinity is essentially the degree of salt concentration. It is also used to define the amount of salt flow. In order to determine the influence of salinity on the decolorization of RBBR, NaCl was utilised in this experiment. *Pleurotus ostreatus* exhibited the highest dye removal rate of 89.89% in the absence of salt (0 mg/L of NaCl) and the lowest dye removal rate of 24.44% when the salinity reached the maximum of 150 mg/L (a drop of 72.81% compared to the dye removal rate in the absence of salt). At a low salt content of 50 mg/L, it was observed that *Pleurotus ostreatus* demonstrated excellent biosorption on RBBR.

Both positively charged sodium cations (Na^+) and negatively charged chloride anions (Cl^-) were introduced into the dye solution by the presence of salt. The chloride anions altered the solution's ionic strength and competed with the dye anions for binding sites on the biosorbent. Chloride anions can also form complexes with the dye cations, which substantially affects the biosorption process. From the standpoint of the activity coefficient, the biosorbent and dye ions tend to be surrounded by a layer of electrostatic interaction. The electrostatic interaction depends considerably on the ionic concentration of the solution. The electrostatic interaction grows as the ionic strength increases. As was previously stated, salinity is directly proportional to ionic strength, and ionic strength is directly proportional to electrostatic interaction. The electrostatic interaction inhibits the dye ions from adhering to the biosorbent's surfaces [27]. In conclusion, electrostatic interaction rises with ionic strength and salinity concentration. Aksu and Balibek [28] discovered that the dried *Rhizopus arrhizus* displayed the maximum biosorption rate on Yellow RL (a type of metal complex in anionic dye) in the absence of salt, but the biosorption rate steadily reduced from 87.2% to 67.0% when 50g/L of sodium chloride was applied. *Trichoderma harzianum*, on the other hand, was able to live in places with a lot of salt, where the biosorption rate was highest (73% at 100g/L) [22].

3.6. Isotherm studies

Compared to Langmuir, Freundlich, and Harkin-Jura isotherms, the biosorption data suited the Jovanovic isotherms the best, as the R^2 value was the closest to 1 at 0.9949 (Table 5). In the Jovanovic isotherm model, the adsorption surface was assumed to be comparable to the estimation for monolayer adsorption without contact. Although Jovanovic and Langmuir models are relatively similar, Jovanovic differs from Langmuir in terms of surface binding vibration. In addition, equilibrium investigations on the biosorption of copper (II) ions by mussel shells done by Farouq and Yousef [29] revealed that the Jovanovic isotherm best fit the biosorption data with the greatest R^2 value (0.9212), when compared to the Langmuir, Freundlich, Temkin, and Elovich models. In contrast, *Aspergillus fumigatus* sp. biosorption of methylene blue dye followed Langmuir isotherm models with the highest R^2 value of 0.9906. Due to the structure of the biosorbent and the dye, it can be deduced that the best-fitted absorption isotherm models for each biosorbent vary [30].

Table 5. Adsorption isotherm of RBBR sorption by *Pleurotus ostreotus*.

Adsorption isotherm	Adsorption constant	Value
Langmuir	q_m (mg/g)	1.12963
	K_L (mg/L)	163.9344
	R^2	0.8981
Freundlich	n	0.665513
	K_F (L/mg)	0.93799387
	R^2	0.875
Harkin-Jura	A	1.6667E-08
	B	1.6667
	R^2	0.9908
Jovanovic	K_j (L/g)	58.539
	Q_{max} (mg/g)	0.00036934
	R^2	0.9949

4. Conclusions

pH affects macrofungi's decolorization of colours. Acidic conditions seemed to be better for decolorizing RBBR by *Pleurotus ostreatus*, but high decolorization was achieved even at pH 7. pH affects macrofungi's decolorization of colours. Acidic conditions seemed to be better for decolorizing RBBR by *Pleurotus ostreatus*, but high decolorization was achieved even at pH 7. After 15 days, the fungus grew rapidly in liquid medium, demonstrating ruderal features. Based on the findings of the trials, the optimal conditions for dye decolorization by *Pleurotus ostreatus* include an acidic pH of 2, the addition of 0.1mL of Tween 80, a sodium chloride concentration of 0 mg/L, and a dosage of 8 plugs. Comparing all of the isotherms, the biosorption data best suited the Jovanovic isotherms in comparison to Langmuir, Freundlich, and Harkin-Jura, with an R^2 value of 0.994.

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Conflicts of Interest

The authors declare no conflict of interest.

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