Efficacy of White Rot Fungal Biomass of *Phanerochaete chrysosporium* for Adsorption and Removal of Reactive Red and Reactive Blue Dyes from Water

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Water pollution has become a worldwide issue, particularly in developing countries. This study aimed to cleanse water contaminated with reactive red (RR-198) and reactive blue (RB-19) dyes *via* adsorption onto nonliving white rot fungal biomass *Phanerochaete chrysosporium* under different operational conditions. It was found that *P. chrysosporium* removed more of RB-19 than RB-198. The conditions of pH 3, temperature 50 °C, adsorbent dosage 0.6 g, particle sizes of adsorbent (0.25 mm), and contact time (30 min) were optimum for removal of the dyes. RR-198 and RB-19 were removed with levels of 78.4% and 86.4%, respectively at pH 3; 79.6% and 90.0%, respectively using 0.6 g of adsorbent dosage; 82.0% and 87.0%, respectively at 30 min; and 82.0% and 87.2%, respectively at 50 °C. Thus, dead *P. chrysosporium* biomass was shown to be a biosorbent for the uptake of RB-19 and RR-198 dyes.

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INTRODUCTION

Numerous artificial dye compounds are harmful, carcinogenic, and mutagenic. Furthermore, dyes have the potential to build up in sediments, particularly in areas where wastewater is discharged, and to disrupt the aquatic system's natural equilibrium. Dye removal can be accomplished *via* adsorption on biomass, biodegradation, or both (Abdel Ghany and Al Abboud 2014; Abdel Ghany *et al.* 2019). Dye biotransformation, as a possible alternative to complete biodegradation to CO_2, *etc.*, may result in less hazardous byproducts. Overexposure to dyes can lead to respiratory issues, skin irritation, and in rare cases, an increased hazard of tumor in humans (Amirza *et al.* 2017; Selim *et al.* 2024). Therefore, it is crucial to successfully remove color from wastewater to ensure that harmful compounds have been removed from treated wastewater before it is released into streams.

Dyes are decolored through a variety of physical and chemical processes, including chemical coagulation, oxidation, ozonation, irradiation, ion exchange, precipitation, and adsorption, as mentioned previously (Makky and Abdel Ghany 2009; Pourfadakari *et al.*

2016). These approaches are not practical because they are costly and have issues with operation. In comparison with other physical and chemical approaches, biological wastewater treatment is frequently the most affordable and environmentally benign option. Because a variety of microorganisms, including algae, yeasts, bacteria, and fungi, are capable of removing different classes of dyes, microbial dye removal techniques, such as bioremoval *via* growing or dead microbial biomass, are frequently used to treat textile industry effluents (Praveen *et al.* 2015). Fungal-based materials contain carbohydrates, amino acids, and fats, along with various chemical groups (hydrocarbons, carboxylic acids, and alcohols) that give them specific characteristics and are used in the process of removing pollutants from water (Ahmed and Ebrahim 2020). *Trichoderma harzianum* was employed to eliminate RR-198, which may be present in liquid effluents from textile factories.

Variables affecting natural environments, such as initial dye concentration, temperature, pH levels, concentration of ions, presence of nutrients, and degradation by sunlight, which can change with the seasons and over time (Aragaw and Bogale 2021), might influence the physical properties and chemical makeup of the dyes. The technician also can adjust the adsorbent dosage, contact duration, and conditions specific to the equipment, such as mixing. Such variables can significantly impact the actual adsorption ability of dye adsorbents. Consequently, dye adsorbents made from biomass need to be thoroughly studied to understand how they absorb specific pollutants.

Several dyes, particularly reactive dyes, which are water soluble), often remain in industrial wastes and thus discharged to the water body. White rot fungi are not producers of mycotoxins (Ganash *et al*. 2016; Al-Rajhi *et al*. 2024), and therefore they are regarded as safe for removal of dyes from water that may be subsequently may use in plant irrigation. So, this investigation aimed to evaluate the removal of RR-198 and RB-19 dyes using the safe white rot fungus biomass at several operating parameters including temperature, pH, adsorbent dosage, initial dye dose, and contact period.

EXPERIMENTAL

Dyes, Reagents, and Fungal Biomass

Two dyes, namely reactive red (RR-198) (formula: $C_{27}H_{18}C_{l}N_7Na_4O_{15}S_5$, molecular weight 967.5 g/mol) and reactive blue (RB-19) (formula: $C_{22}H_{16}O_{11}N_2S_3Na_2$, molecular weight 626.5 g/mol), were employed in the present experiments and were supplied by Sigma Company. The additional chemicals utilized in these tests were from Merck Company (India). Throughout the investigation, double-distilled H_2O was utilized to dissolve the dyes and to make stock solutions. The stock solution was diluted in the proper ratios to create dye solutions with varying starting concentrations. White rot fungus, namely *Phanerochaete chrysosporium*, was taken from Prof. Abdelghany T.M.

White Rot Fungus Biomass as Dyes Adsorbent

The white rot fungus was washed with distilled H_2O to eliminate the dirt and dust particles, dried in air, and subsequently placed in an oven at 85 °C for one day until constant weight was obtained. The fungal biomass was crushed using a mortar and pestle, and then it was sieved to get different sizes of dried biomass particles including 0.25 and 0.5 mm used as dyes adsorbent.

Effect of Different Conditions on Dyes Removal

The adsorption amounts and rates were determined using the batch mode of operation. The influences of investigational parameters for the kinetic experiments namely temperature (10 to 60 °C), initial dye concentration (10 to 160 mg/L), pH (3 to 9), and contact time (5 to 35 min), adsorbent dosage (0.2 to 1.4 g/L), and particle size of adsorbent (0.25 and 0.5 mm) on the level of dyes adsorption were investigated. For adjusting the solution pH, NaOH (0.1 mol/L) and HCl (0.1 mol/L) were employed. Using a UV spectrophotometer (IRMECO of Model U2020), the levels of RR-198 and RB-19 adsorptions were recorded at wavelengths of 520 nm and 592 nm, respectively.

Removal efficacy (RE) of dyes was recorded using Eq. 1:

$$\text{RE (\%)} = \left[\frac{\text{Initial concentration} - \text{Final concentration}}{\text{Initial concentration}}\right] \times 100 \qquad (1)$$

Statistical Examination

The mean of the three results of every experiment was used to calculate the standard deviation (± SD). The software Graph Pad Prism V5 (San Diego, CA, USA) was utilized to assessment the outcomes for one-way of variance (ANOVA).

RESULTS AND DISCUSSION

The nonviable white rot fungal biomass *Phanerochaete chrysosporium* was applied to remove the reactive dyes, namely RR-198 and RB-19 (Fig. 1). From the obtained results, the removal of dyes was favoured by temperature increase up to 50 °C. The level of dyes removal was improved from 17.6% to 82.0% for RR-198; from 19.6% to 87.2% for RB-19 by changing the temperature from 10 to 50 °C, respectively. The high temperature may affect the internal structures of biomass and thereby enhance the penetration of dyes. The findings from this study revealed that adsorption of the dyes *via* fungal biomass was regulated by endothermic progressions. Changes in temperature primarily affect the rate of adsorption because they can accelerate the diffusion of adsorbent molecules through the outer layer and the internal pores of sorbent compound particles by reducing the viscosity of the solution. Moreover, for a given adsorbate, a temperature shift may also increase the adsorbent's propensity to remove the dyes.

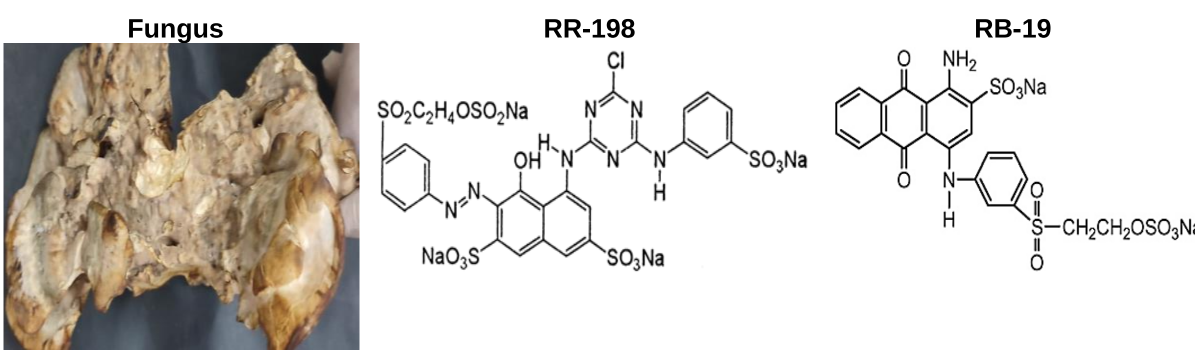

Fig. 1. Biomass of used fungus and chemical structure of used dyes

Based on the review article of Badawi *et al*. (2021), the adsorption rate of methylene blue is temperature dependent. Dead fungal biomasses of *Saccharomyces* *cerevisiae*, *Rhizopus nigricans*, *R. arrhizus*, *Aspergillus japonica*, and *A. niger* as well as white rot fungi *Pycnoporous sanguineus* and *Phanerochaete chrysosporium* were applied for removal of reactive dyes including brown 9, black 8, green 19, blue 3, and blue 38 with highest adsorption using *S. cerevisiae* at 30 °C and *R. nigricans* at 20 °C (Kumari and Abraham 2007) compared to white rot fungi. *Aspergillus fumigatus* was applied in dead and live form for degradation of Congo red, the degradation increased within temperature increasing up to 30 °C, and then slowly declined at 40 °C, while at 50 °C it severely affected the dye degradation (Abdel-Ghany *et al*. 2019). The differences among the results of these studies and the present study may be due to used adsorbent type, the cell wall structure, and using the adsorbent in dead or live form.

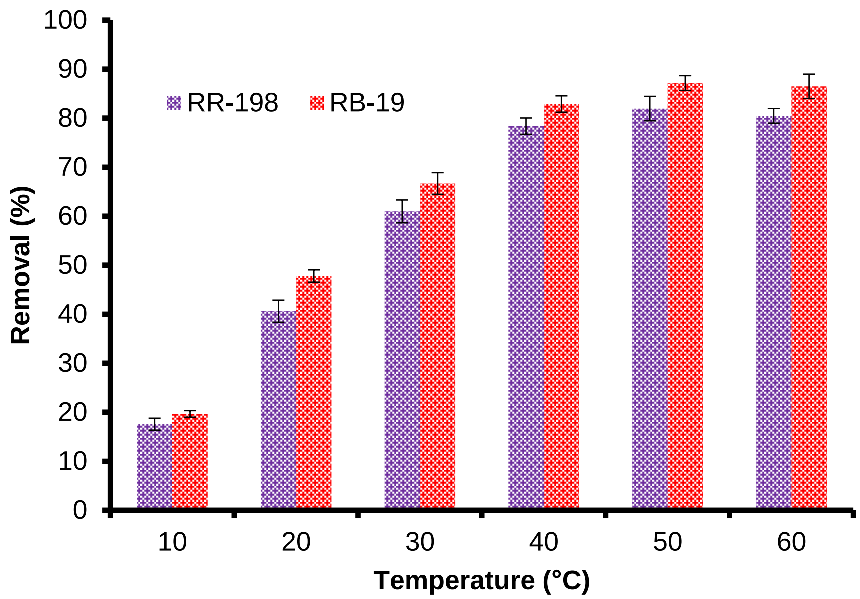

Fig. 2. Removal of RR-198 and RB-19 dyes at different temperature by fungal biomass

The adsorptive elimination of dyes from aqueous solutions is significantly influenced by pH, which also controls the adsorbent's surface charge and the level to which the components in the solution are ionized. Ionic dyes are present in the aqueous system. Because they are a charged species, the biosorbent's surface charge, which is determined by the pH of the solution, determines how much of them absorb onto the surface. The characteristics of biomass materials, as well as the adsorption processes and dye molecule dissociation, are affected by the pH of the substrate. The findings demonstrated that at low pH 3, both dyes RR-198 and RB-19 displayed their maximal biosorption (Fig. 1). Lower pH levels resulted in a positively charged biosorbent surface and the development of electrostatic interaction among positively charged biomass and negatively charged anionic dyes. However, hydroxyl ions, which compete with dye anions for binding sites, cause adsorption to decrease at basic pH. A previous study reported that low pH 2 was the optimum for RR-198 removal by biomass of *Aspergillus parasiticus* (Akar *et al*. 2009). The reduction in the ability of the biosorbent to absorb dyes as the pH of the solution rises can be understood by considering the dye attachment sites on the biosorbent biomass. These sites become negatively charged, which makes it harder for dye molecules to bind to the material due to electrostatic repulsion. Ahmed and Ebrahim (2020) cleared that a low pH of 2 increased anionic dyes removal, unlike cationic dyes. Ruscasso *et al.* (2021)

reported 50% removal of RB-19 using biomass of *Debaryomyces hansenii* at pH 6. Additionally, *Panus tigrinus* (white-rot fungus) exhibited the highest decolorization (83.18%) of azo dyes at pH 2 (Mustafa *et al*. 2017). Furthermore, Elumalai *et al.* (2023) demonstrated that pH 3 was effective among other pH levels on the removal of RY 186.

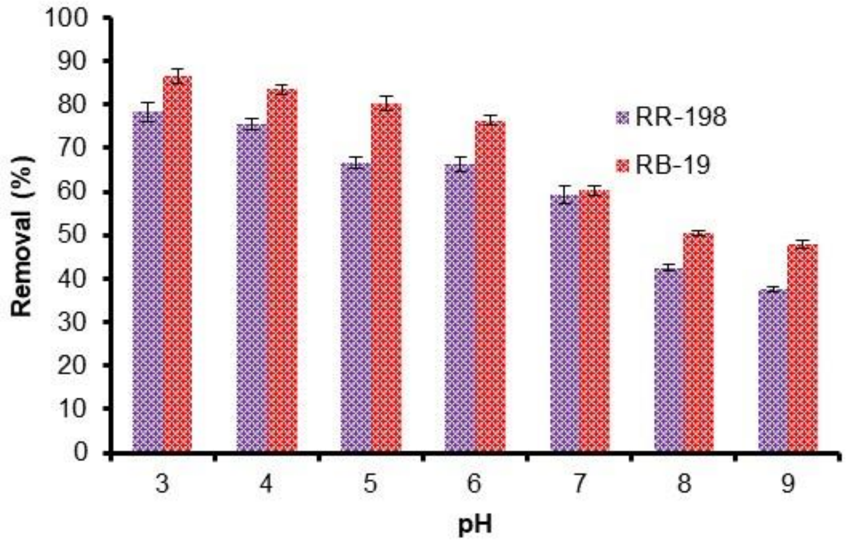

Fig. 3. Removal of RR-198 and RB-19 dyes at different pH by fungal biomass

Figure 2 illustrates the influence of varying adsorbent dosages (0.2 to 1.4 g/L solution) on dye removal under the same operating circumstances. Within 30 min of adsorption, it was shown that removal increased significantly at larger adsorbent doses. Maximum removal of RR-198 and RB-19 was observed with levels of 79.6% and 90.0%, respectively, at 0.6 g of adsorbent dosage, and then the removal percentage decreased with increasing the adsorbent dosage. However, the high dose of adsorbent above 0.6 g was more efficient than that below optimum adsorbent dosage. For instance, at 0.4 g, the removal level was 42.5% and 45.7%, while at 0.8 g the removal was 79.4% and 88.4% for RR-198 and RB-19, respectively. This observation may be attributed to the available area of adsorbent for adsorption, which increased with the biomass increment. However, the continuous decline of current dyes in the solution was one of the reasons for declined removal at high dosage of the adsorbent.

The optimum suitable adsorbent dosage may depend on type of used dyes and biomass. For example, *Rhizopus arrhizus* at 4 g/L was the optimum for maximal removal (99.5%) of Everzol Black (EB) from aqueous solution (Gül and Silah 2014). Hassan *et al*. (2024) observed that *Yarrowia lipolytica* biomass ranging from 5 to 300 mg increased the removal of acid red 18, and reactive yellow 18, in addition to basic blue 41. According to Loai (2018), the limit of dyes adsorption occurs when most of the adsorption locations are overloaded because of the large amount of adsorbent, providing a constant rate of dyes removal. According to previous study, Kabbout and Taha (2014) mentioned that the kinetic of biosorption of methylene blue by dead biomass of *Aspergillus fumigatus* is directly linked to the biosorbent surface. Moreover, the particle size represents an important factor in the biosorption process. In the present investigation, the biomass in crushed form (size 0.25 and 0.5 mm) was effective in the biosorption of RR-198 and RB-19 (74.5 and 82.3 %) compared with bulk biomass (23.5% and 33.2%) (Fig. 5). The present results match with other results (Santhi *et al.* 2009), indicating that small sizes of biosorbent encouraged the dyes adsorption.

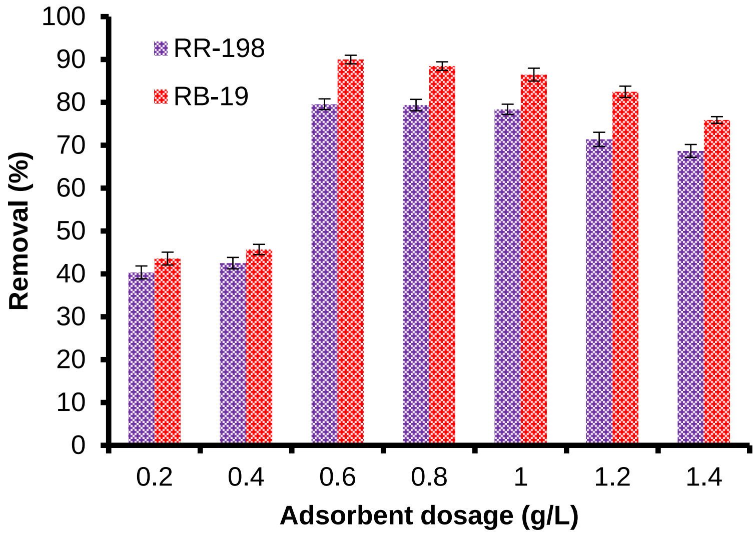

Fig. 4. Removal of RR-198 and RB-19 dyes at different adsorbent dosages of fungal biomass

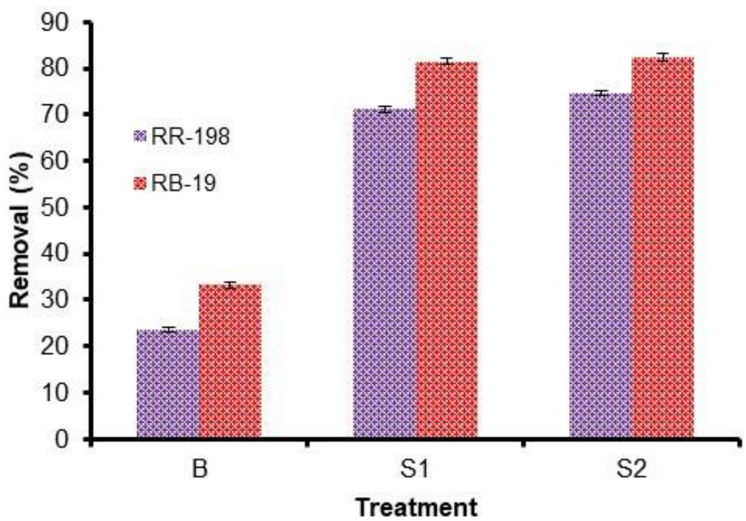

Fig. 5. Removal of RR-198 and RB-19 dyes at different adsorbent sizes of fungal biomass. B (bulk biomass without Crushing), S1 (size: 0.5 mm), and S2 (size: 0.25 mm)

The efficacy of contact time on the removal of RR-198 and RB19 dyes was studied and is shown in Fig. 6. Range of contact time (5 min to 35 min) was applied to calculate the dyes removal at each studied period employing 0.6 g biomass. The removal percentage increased with increasing contact time up to 30 min, but at 35 min the removal percentage decreased. At 30 min, the removal level was 82.0% and 87.0%, while at 35 min the removal was 81.9% and 87.0% for dyes of RR-198 and RB19, respectively. Next adsorption, the uptake rate of dye is limited by the level of dyes transportation from the outside to the inside locates of the adsorbent biomass. The effect of contact time on the removal of RR-198 dye was investigated at 10 min up to 150 min, the investigation indicated that the removal level increased at initial periods up to 60 min, and subsequently declined gradually (Ashna and Heydari 2020). At the initial time the adsorbent surface may be available to adsorb the dyes but then the surface becomes saturated and there is difficult to join by the dyes. The contact time (90 min) was the optimum period for decolorization of azo dyes by *P. tigrinus* (Mustafa *et al.* 2017).

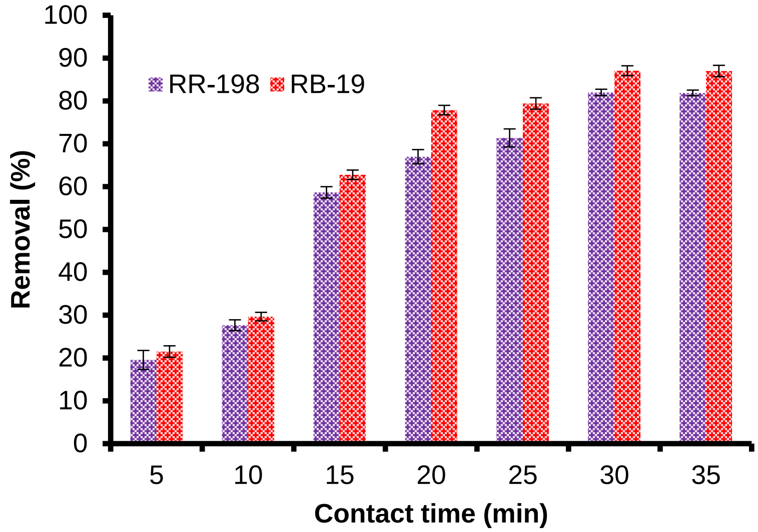

Fig. 6. Removal of RR-198 and RB-19 dyes at different contact times by fungal biomass

CONCLUSIONS

1. The non-viable biomass of *Phanerochaete chrysosporium* showed excellent adsorption capacity to remove Reactive Red 198 (RR-198) and Reactive Blue 19 (RB-19) dyes.
2. Temperature (50 °C), solution pH (3), and contact time (30 min) in addition to adsorbent dosage (0.6 g) and particle size of adsorbent (0.25 mm) were critical factors for removal of reactive dyes from aqueous solutions.

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