Effectiveness of Using *Trichoderma viride* as Biosorbent for Remazol Brilliant Violet in Batik Wastewater Treatment

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Submission: April 2020; **Revised:** May 2020; **Accepted:** May 2020

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ABSTRACT. Synthetic dyes used in the batik industry cause negative impacts on the environment. One type of synthetic dyes used in batik industry is remazol brilliant violet. This synthetic dye is reactive, carcinogenic, and non-biodegradable. Therefore, a strategy to degrade this synthetic dye is needed. In this study, the potential application of *Trichoderma viride* for biosorption of remazol brilliant violet (RBZ) in batik wastewater was investigated. The current work aimed on determination of the optimum conditions, including pH, contact time, and RBZ concentration. Biosorption of RBZ was carried out at pH 4 to 9; with contact times of 12, 24, 30, 36, and 42 h, with concentrations of dye solutions were 40, 60, 70, 80, and 90 mg/L. The results showed that the optimum biosorption of RBZ was obtained at pH 4, for 30 h, and the optimum concentration of RBZ at 60 mg/L, with the RBZ absorbed was 79.27%. The FTIR analysis suggested that RBZ biosorption changed the chemical functional groups in the *T. viride*. The FTIR spectra revealed that the biomolecules that were affected during the process of biosorption were mainly lipids and proteins, and slightly from nucleic acids and carbohydrates.

Keywords: Biosorption, FTIR analysis, RBZ, *Trichoderma viride*

INTRODUCTION

Batik is one of the works of Indonesian art that still exists and continues to be used. Since batik has been selected as one of the Masterpieces of the Oral and Intangible Heritage of Humanity by UNESCO on October 2, 2009, the batik industry is increasingly favored by Indonesian and also foreign communities. There are currently around 4.5 million batik industry businesses in Indonesia [1]. Most batik industries use synthetic dyes because they are affordable, durable, and easy to use. However, artificial chemical dyes usually are large complex compound that cause them difficult to degrade and can persist in the environment for a long time period [2].

One of the synthetic dyes that are widely used in the batik industry is remazol dyes. Remazol violet 5R is a reactive dye from the azo group [3]. Azo dyes are very reactive and do not fade easily because they have chromophore groups (as color carriers) and auxochrome groups (as color binders). In addition, this dye also has a stable aromatic complex structure that is resistant to chemical oxidation reactions [3]. This dye can be harmful, as

it causes skin irritation, affect to the respiratory system and digestive tract [4].

The methods used to reduce the intensity of dyes in wastewater include coagulation, filtration, electro-decolorization, flocculation, and coagulation [5, 6]. Nonetheless, these applications result in a vast chemical sludge production or non-economical to operate. Therefore, it is essential to protect the water reserves by searching a green and reasonable solution.

Biosorption is the latest wastewater treatment technology that can remove metals and toxic dyes [7]. The biosorption process can occur due to the presence of biological materials called biosorbent and the presence of a solution with high affinity, thus, this is easily bound to biosorbents. Biosorbents used can be fungi, bacteria, algae and biopolymers [8].

Decolorization of dyes can use fungi, since fungi produce extracellular ligninolytic enzymes that are able to remove aromatic compounds and degrade various organic and inorganic contaminants [7]. Studies that have been carried out using fungi as a dye biosorbent include using *Coriolus versicolor* for decolorization of brilliant

violet remazol [9] and *Aspergillus sp* for indigosol blue-04B biodegradation [10].

In a previous study [11], the use of immobilized *T. viride* on Ca-alginate for RBZ biosorption has been applied. The biosorption process resulted in the optimum condition of 60 min, pH 4, with percent of adsorption was 8.76%. In this study, *T. viride* is directly used to conduct biosorption process of RBZ dye, without immobilization. Therefore, in particular, the objectives of the present work are to determine the influences of time, pH, and concentration on the RBZ biosorption process using *T. viride*.

METHODS

Trichoderma viride Cultivation

PDA (potatoes dextrose agar) from Sigma-Aldrich was used as a solid growth medium for *T. viride* sub-culturing and adaptation. A loop full of the culture of *T. viride* was cultivated in the sterilized PDA incubated for 6-8 days, at 37°C. Subsequently, *T. viride* spores appeared in the PDA media was dispersed in 1-2 mL of sterilized bi-distilled water. These spores were then moved to a broth growth medium (100 mL) in 250-mL Erlenmeyer flasks on a rotary shaker, and grown at ambient temperature, at a speed of 150 rpm, for 40 h. The suspension collected as *T. viride* inoculum and will be used for the biosorption process.

The Biosorption Process of RBZ

A six-solution of 40 mg/L RBV was adjusted to pH 4, 5, 6, 7, 8, and 9 respectively using a pH meter. The pH was accustomed by the 0.1 M HCl or 0.1 M NaOH addition into those solutions. A 25 mL of 40 mg/L RBV solution with pH of 4, 5, 6, 7, 8, and 9 were contacted with 20.0 mL *T. viride* inoculum. Then the solution mixture was incubated until the mid-logarithmic phase (36 h). For the time influence, the biosorption process was conducted as follows, a total of 25 mL of 40 mg/L ppm RBV solution with optimum pH was contacted with 20.0 mL of *T. viride* inoculum. The biosorption process was conducted into the time variations of 12 h, 24 h, 30 h, 36 h, and 42 h.

For the concentration variation, biosorption process was applied in the same methods, using various RBZ concentrations of 40, 60, 70, 80, and 90 mg/L, in the optimum pH and time contact time, using a 20 mL *T. viride* inoculum. Next, after all the biosorption process finished, the solution was filtered using Whatman filter paper no. 40 to separate *T. viride* from the filtrate. The resulted filtrate was then measured its absorbance using a UV-visible spectrophotometer (1601-Shimadzu). The resulted solid from the filtration process was

kept and then was analyzed by FTIR spectrophotometer (8400S/Shimadzu). All process was conducted in triplicates. The calculation of the number of RBZ during the biosorption process was determined using this equation:

$$\% \text{ of biosorption} = [(Ca - Cb)/Ca] \times 100\% \quad (1)$$

where

Ca = concentration before biosorption (mg/L)

Cb = concentration after biosorption (mg/L).

Statistical Analysis

Data was analyzed using the ANOVA with a level of confidence of $\alpha = 0.05$, and the differences between variation with student's T test.

Figure 1. The chemical structure of remazol brilliant violet dye.

RESULTS AND DISCUSSIONS

The effect of pH on the biosorption process of RBZ on *T. viride* is shown in Figure 2. There was a decrease from pH 4 to pH 9, in the biosorption of RBZ. In the acidic condition or at low pH, the biosorbent surfaces are protonated, since H^+ ion is abundant in acidic condition. The pH of the medium affects both the solubility of RBZ and ionization of the functional chemical groups including $-COO^-$, $-OPO_3^{4-}$, and $-NH^-$, of the cell walls of *T. viride* [12]. These functional groups have negative charges, and thus, they are acidic. Moreover, in the low pH, less than 7, the protonated adsorbent can result in the electrostatic interaction between adsorbent and the RBZ; considering RBZ has a negative $-S$ group and hence, is an anionic dye.

Figure 2. The RBZ biosorption in the pH variations of pH 4 to PH 9.

A previous study of RBV biosorption using periwinkle shell resulted in decreasing adsorption along with increasing of the pH [10]. Another study by Rapo, *et. al.*, 2019 [3] on the RBZ biosorption using calcinated egg skin resulted in the highest adsorption rate at pH 4. These studies were in agreement with the results of our current work, that at the low pH causing the adsorbent surfaces were surrounded by protonated H^+ ion. The statistical analysis showed that from pH 5 to pH 9, the adsorption percentage was not different significantly ($p < 0.05$). Hence, pH 4 was chosen as the optimum pH for the biosorption process, with the number of RBZ adsorbed was 79.05%.

Figure 3. The RBZ biosorption in the time variations of 12 to 42 h.

Figure 4. The RBZ biosorption in the variations of RBZ concentration of 40 to 90 mg/L.

Biosorption of the dye increased from 12 to 30 h, then the number of RBZ adsorbed declined from 30 to the end of the process. As a result, the optimum time for biosorption process was 30 h, with the RBZ adsorbed was at 93.42% (Figure 3). The RBZ biosorption experiment was a two-steps adsorption. At first, biosorption occurred fast, because the active sites of the adsorbent were unoccupied, from 12 to 30 h. Next, from 30 to 42 h, the balance of adsorption and desorption reached. Consequently, the biosorption rate

decreased, due to the active sites were already fulfilled with the dye. It was suggested that RBZ biosorption was as a result of simply physico-chemical interactions between *T. viride* and RBZ solution [13]. In addition, the kinetics of RBZ biosorption involved no energy-mediated reactions.

On the variations of RBZ concentration, percent adsorption was 76.71% in a solution of 40 mg/L. Then at a concentration of 60 mg/L RBZ adsorption was 79.27%, at concentrations of 70, 80, and 90 mg/L percent of adsorption were 79.22%, 76.99%, and 75.43%, respectively (Figure 4). From Figure 4, it was shown that from the concentration of 40 to 60 mg/L the number of RBZ adsorbed increased; then decreased at the concentration of 70 to 90 mg/L. The difference in the amount of RBZ adsorption related to the toxicity of the dyes. The toxicity of azo dyes increases with increasing concentration. Therefore, the rate of adsorption keeps increasing to a certain concentration; and subsequently decreases with increasing dye toxicity [14]. However, statistical analysis results revealed that there were no significant differences between variations in the concentration of 40 to 90 mg/L, ppm because they had the same notation. Consequently, the RBZ concentration did not affect to the biosorption process.

Results from FTIR analysis are shown in Figure 5, and the assignment of the band of interest is listed in Table 1. It is shown in Figure 5 that there were no significant wavenumber shifts. According to Ratnawati *et.al.* (2010) [15] proteins and polysaccharides play an important role in the process of ion biosorption where covalent bonds occur including amino and carboxyl groups. Before biosorption process, there was absorption band at 1649.02 cm^{-1} indicated the presence of the $C=O$ amide group on *T. viride*. Another band that appeared at 1546.80 cm^{-1} indicated the presence of $-NH$ from amide group. At a wavenumber 3429.20 cm^{-1} which shows the absorption band of the $O-H$ from carboxylic group. This group is a signature of proteins. After the biosorption, there was a wavenumber at 1652.88 cm^{-1} , assigned as the $C=O$ carbonyl, and this band was supported by the absorption band at a wave number of 1652.88 cm^{-1} , indicating the presence of $-N-H$ from amide group. Finally, a wavenumber at 3402.20 cm^{-1} , which shows the absorption band of the carboxylic $O-H$ group. *T. viride* that have been contacted with RBV analyzed by FTIR showed changes in intensity in the absorption bands, but there were still the same functional groups as *T. viride*. From the FTIR analysis, it is suggested that the biosorption of RBV did not damage the structure of *T. viride*.

Figure 5. The FTIR spectra *T. viride* before (black line) and after (red line) biosorption process of 40 mg/L RBZ at pH 4, for 30 h, at ambient temperature.

Table 1. Analysis of functional groups in *T. viride* before and after biosorption of RBZ

No.	Before Biosorption		After Biosorption	
	Wavenumber (cm ⁻¹)	Designation	Wavenumber (cm ⁻¹)	Designation
1	3429.20	O-H Carboxylate	3402.20	O-H Carboxylate
2	2923.38	C-H alkene	2927.74	C-H alkene
3	1649.02	C=O amide	1652.88	C=O amide
4	1546.80	N-H amide	1544.88	N-H amide
5	1083.92	C-N amide	1076.21	C-N amide

CONCLUSION

The current work has shown that the biosorption process of RBZ using *T. viride* was affected by pH and time. In this study, optimum conditions were achieved at pH 4 with a contact time of 30 h, and at a concentration of dye solution of 40 mg/L, with percent adsorption was 76.27%. The use of *T. viride* for the biosorption process of synthetic dye RBZ can be used as one of the green techniques for synthetic dye degradation in batik wastewater.

ACKNOWLEDGEMENT

Author thank to the facility that provided by the Biochemistry Laboratory, Chemistry Department, Brawijaya University.

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