Biosorption of Synthetic Dye from Batik Wastewater Using *Trichoderma viride* Immobilized on Ca-Alginate

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**Abstract.** Batik and textile industry is one of the sources of liquid waste derived from the staining process. One of the color substances commonly used is remazol brilliant violet which is an azo-type substance that is difficult to be degraded in the environment and is toxic pollutants. A method that can be used to decolorize of synthetic dye is biosorption. *Trichoderma viride* can be used as biosorbent, since it is resistant to toxic compound. The immobilization of *T. viride* is applied to increase their stability and to minimize cellular damage. Calcium alginate can be used for immobilization matrix because it is affordable, non-toxic and biocompatible. This study focused on determining the optimum conditions on biosorption process of remazol brilliant violet using immobilized *T. viride*. Remazol brilliant violet solution were absorbed at pH 4, 5, 6, 7, 8, and 9, contact time for 10, 20, 30, 50, 60, 70, and 90 min, and the amount of adsorbent used were varied at 0.3; 0.4; 0.6; 0.8; and 1 g. The optimum condition was obtained at 60 min biosorption time, at pH 4 with the amount of adsorbent was 1 g, with percent of adsorption was 8.76%. The adsorption capacity of remazol brilliant violet using *T. viride* immobilized with Ca-alginate was 0.089 mg/g. FTIR spectra results indicated functional groups that involved in immobilization of *T. viride* were O-H and C=O carboxylic, while in biosorption process violet were C-H sp³, C=O carboxylc, C=O amide and C=C benzene.

**Keywords:** biosorption, remazol brilliant violet, *Trichoderma viride*, immobilization, Ca-alginate

1. **Introduction**

At the present time, batik industry has become popular and has many contributions to the economic growth for Indonesia. Batik industrial is mostly manufactured in a small scale industry. Nonetheless, awareness on the importance of clean practices in the production of batik is degrading. This is because, some of the batik manufacturers release batik waste into water directly without proper pre-treatment. It is known that batik industries use large amount of synthetic dyes during their coloring process [1]. As a result, the presence of synthetic dyes in the wastewaters leads to harsh environmental pollution and also cause serious health risks, since synthetic chemical dyes have a complex molecular structure which makes them more stable, and can stay in the environment for a prolonged time period [2].

Because of this serious water pollution, there is persistent need from the researchers to find an alternative and friendly method to preserve the environment. At this time, there are many technologies available for the solution to the problems caused by the textile industry, including chemical precipitation, ultrafiltration, carbon adsorption, coagulation, flocculation and ozone oxidation [3]. Nevertheless, these
treatments were either non-economical to apply, or create a huge chemical sludge production [3]. Thus, it is important to protect the water resources by seeking more environmental friendly and affordable solution to solve this problem.

Remazol brilliant violet (RBZ) is frequently used in textile industry, including batik home industry. This synthetic dye has aromatic azo groups (-N=N-) [4]. These azo groups make this molecule non-degradable. Decolorization of synthetic dyes, therefore, is important. The biosorption process of synthetic dyes can be performed with the use of microorganisms, fungi, or yeast, i.e. Trichoderma viride [5]. However, direct use of these microorganisms have some drawbacks, such as they are unstable and easily damage due to extreme environment. Thus, the immobilization is conducted to increase the surface of the fungi, to improve fungi resistant from the environmental stress live in toxic environment [6]. Cell entrapment in Ca-alginate is one the immobilization methods. The entrapment method with Ca-alginate offers many benefits, including this is easy to apply, economical, and bio-degradable [7].

Therefore, this study investigates the efficiency of immobilized T. viride on decolorizing of batik wastewater model, using RBZ dye. In addition, in the biological adsorption processes, numerous physicochemical parameters including pH, temperature, oxygen, time period, dye structure and dye concentration, directly influence the capability of bacterial decolorization of dye wastewater and also effects on microbial growth [8]. Therefore, in particular, the aims of the current study are to investigate the effects of pH, contact time, and the amount of biosorbent on the biosorption process of RBZ on immobilized T. viride.

2. Materials and Methods

2.1. Chemicals and Instrumentation

Materials used in this study were culture of Trichoderma viride obtained from microbial stock collection of Microbiology Laboratory, Universitas Negeri Malang. All reagents of analytical or higher purity grade were purchased from Merck or Sigma-Aldrich and were used as received: HCl (37% aqueous solution), HNO₃ (trace pure, 65% w/w in H₂O), NaOH (99.9%), H₂SO₄ (98%, d = 1.84 g/mL), ethanol (96%), CaCl₂·2H₂O, KH₂PO₄, remazol brilliant violet, Na-alginate. Water was purified using distillation technique. The growth media for was potatoes dextrose agar (PDA). The determination of remazol brilliant violet concentration before and after biosorption process was conducted using UV-Vis spectrophotometer (1601-Shimadzu). The functional groups change in the biosorbent before and after biosorption process was determined using FTIR spectrophotometer (8400S/Shimadzu).

2.2. Immobilization Process of T. viride with Ca-alginate

A 1.25 g of Na-alginate was added to 80 mL of liquid media, heated and stirred with magnetic stirrer. Cultures of T. viride growth in solid media was harvested with sterile water. These were mixed with a mixture of Na-alginate and liquid media. The mixture was shaken for 36 h in room temperature. The solution mixture was added to 20 mL of 0.15 M CaCl₂ solution drop wise, until the biosorbent beads were formed.

2.3. Biosorption Process of Remazol Brilliant Violet with Immobilized T. viride

A 25 mL of RBZ solution (40 mg/L) was transferred into Erlenmeyer flask. A 0.4 g of immobilized T. viride was added as biosorbent. Biosorption process was carried out at room temperature, pH 4, in variations of contact time for 10, 20, 30, 50, 60, 70, and 90 min. For the pH influence, the biosorption process was conducted in similar ways, the pH was varied at pH 4, 5, 6, 7, 8, and 9, in 60 min of time contact. All experiment was conducted in triplicates. Finally, the amount of biosorbent used were determined in the variations of 0.3, 0.4, 0.6, 0.8, and 1 g. The concentration of RBZ was calculated after biosorption process. Determination of the amount of RBZ after biosorption process was calculated using this formula:

Biosorption (%) = ((Co-Ce)/Co x 100%), where Co = initial concentration (mg/L), Ce=final concentration (mg/L). Adsorption capacity of immobilized T. viride was determined as follow:
Qt = (Co-Ce) V/W; where Qt = adsorption capacity (mg/g), Co = final concentration (mg/L), Ce = final concentration (mg/L), V = sample volume (L), and W = adsorben mass (g). The precipitates from biosorption process were collected and analyzed using FTIR spectrophotometer at a range of 4000-400 cm⁻¹ (8400S/Shimadzu).

3. Results and Discussion

Figure 1 shows the effect of time on the biosorption process of RBZ on immobilized T. viride. Biosorption of the dye increased time by time, since the beginning of the process (10 min), until at the end of the assay (90 min). However, statistical analysis revealed that from 60 min onward, the percentage of adsorption was not significantly different (p<0.05). Therefore, 60 min was the optimum contact time for the biosorption process, with the amount of RBZ adsorbed was 9.4%. The biosorption process of RBZ was found to be in a two-stages. In the beginning, biosorption process was fast, since the active sites of the adsorbent were still available to adsorp the dye, from 10 to 60 min. After that, 60 to 90 min, the equilibrium of adsorption and desorption was achieved. Therefore, the biosorption process started to slowing down, due the active sites were already occupied with the dye. The biosorption kinetics observed in biosorption process is proposed by involving no energy-mediated reactions and dye removal from solution is due to purely physico-chemical interactions between microorganism and dye solution [9].

![Figure 1](image-url)

**Figure 1.** Result of biosorption of remazol brilliant violet using immobilized T. viride, under the influence of time (10, 20, 30, 50, 60, 70, and 90 min).

The influence of pH (pH 4, 5, 6, 7, 8, and 9) on the biosorption process of RBZ is shown in Figure 2. Interestingly, from the lowest pH used, pH 4, to the highest pH, pH 9, the adsorption of RBZ showed a decrease. This is because at low pH, surface of the biosorbent has been protonated, due to the abundance of H⁺ ion in acidic condition. The pH of the medium affects both the solubility of RBZ and ionization of the functional groups such as –COO⁻, -PO₄³⁻, and –NH⁺, of the cell wall of T. viride; which are acidic and carry negative charges rendering cell walls [10]. At acidic pH, the protonated adsorbent resulted in electrostatic interaction between the dye and adsorbent, since RBZ is anionic dye that has negative –S group. This result is in agreement with the previous study by Bello. et. al. [4], that investigated adsorption of synthetic dye using activated carbon, the adsorption of the dye decreased as
the pH increased. In another study [11], RBZ adsorption using rice hulls resulted in the highest adsorption at acidic pH, pH 1.

![Figure 2](image1.png)

**Figure 2.** Result of biosorption of remazol brilliant violet using immobilized *T. viride*, under the influence of pH.

![Figure 3](image2.png)

**Figure 3.** Result of biosorption of remazol brilliant violet using immobilized *T. viride*, under the influence of mass of adsorbent.

Effect of the amount of biosorbent (immobilized *T. viride*) was investigated by contacting RBZ and *T. viride* with previous optimum conditions that obtained before (pH 4, contact time 60 min), with the amount of biosorbent ranging from 0.3 to 1 g. It was found that the amount of RBZ taken up by the immobilized *T. viride* increased slowly with an increase of the amount of biosorbent from 0.3 to 0.6 g.
A rapid increase was observed when the amount of biosorbent greater than 0.6 g. The highest amount of biosorbent used, 1 g, resulted in the highest amount of RBZ adsorbed, at 8.76%. In this study, the percent of adsorption was not optimal (less than 50%). There are several reasons for this. Firstly, the pores size of Ca-alginate used to immobilize T. viride are relatively small, 5.2 nm ± 0.9 nm [12]. Moreover, the molecular weight of RBZ is remazol brilliant violet is a bulky molecule. Based on National Center for Biotechnology Information [13], RBZ has relatively high molecular weight of 735.563 g/mol.

Adsorption capacity of the immobilized T. viride was calculated at optimum conditions, at pH 4, time contact of 60 min, and the amount of adsorbent was 1 g. The adsorption capacity obtained indicates the capacity of immobilized T. viride can absorb RBZ in those conditions. In this study, the adsorption capacity was 0.089 mg/g. In contrast, a study conducted by Bishnoi et. al. [14] reported biosorption of Cr(VI) metal using T. viride which was immobilized with Ca-alginate, that resulted in higher adsorption capacity, 16.075 mg/g. The differences may be caused by the size of Cr(VI) metal ions which are smaller than RBZ.

The analysis of functional group involved in the biosorption process was conducted using FTIR spectrophotometry. Figure 4 shows the FTIR spectra of immobilized T. viride before and after biosorption process, while assignment of the bands of interest is listed in Table 1.

![Figure 4. The FTIR spectra from immobilized T. viride before and after biosorption process of remazol brilliant violet, at pH 4 and contact time of 60 min.](image)

### Table 1. FTIR spectra assignment of bands of interests from Figure 4.

| No | Wavenumber (cm⁻¹)      | Assignment [15, 16]   |
|----|------------------------|-----------------------|
|    | Before biosorption     | After biosorption     |
| 1  | 3423.41                | 3452.34              | -O-H carboxylate          |
| 2  | 2929.67                | 2929.67              | -C-H sp³                   |
| 3  | 1631.67                | 1643.24              | -C=O amide                 |
| 4  | 1533.30                | 1533.30              | -C=C aromatics rings      |

FTIR spectra of T. viride (non-immobilized) have been reported in our previous study [rensani paper]. In the FTIR spectra of immobilized T. viride, there were only appeared two bands of interest, at around 3430 cm⁻¹, assigned as -O-H stretching from carboxylate groups, and ~1630 cm⁻¹, assigned as C=O amide, that may come from functional groups in Ca-alginate. The difference between FTIR soectra of Ca-alginate and immobilized T. viride is that in the -O-H alcohol band of T. viride there is an overlapping...
band of N-H amide. This can be seen from the FTIR spectra of immobilized T. viride which has a shoulder in the wavenumber at around 3500 cm⁻¹.

After biosorption process, there was band at ~2930 cm⁻¹, indication of -C-H sp3. In addition, there were bands at wavenumber around 1643 cm⁻¹ and 1530 cm⁻¹ from C=C benzene or aromatic rings from the RBZ. The functional groups of C=C aromatics have specific two bands at around 1600 cm⁻¹ and 1500-1450 cm⁻¹. The C=C aromatics bands indicated that there was bond formation between RBZ and immobilized T. viride. The bond formation is from positively charged amide group from T. viride with sulphonate group from RBZ. This bond may also cause a shift in wavenumber and decrease in percent transmittance (%T).

4. Conclusion
This study has demonstrated that biosorption process of immobilized T. viride on Ca-alginate is affected by time, pH, and the amount of biosorbent. The optimum conditions achieved in the study were at pH 4, at 60 min, and 1 g of biosorbent mass, with the percent of adsorption at 8.76%. The adsorption capacity was 0.089 mg/g. The functional groups involved in the biosorption process were -C-H sp3, C=O amide, and -C=C benzene. In order to increase adsorption capacity, the use of natural polymers or matrices that have larger pore size is needed, since the synthetic dye is generally has large molecular weight. The biosorption process of RBZ using immobilized T. viride can be applied as one of alternative solutions for synthetic dye removal in the environment.

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