

Biosorption of Cr(VI) in Aqueous Solution using Microorganisms: Comparison of the Use of *Rhizopus oryzae*, *Bacillus firmus*, and *Trichoderma viride*

Anna Safitri^{1*}, Putri Mahardini¹, Sasangka Prasetyawan¹, Anna Roosdiana¹

¹Department of Chemistry, Brawijaya University, Malang

Email: a.safitri@ub.ac.id

Abstract. In this work, the study of biosorption of Cr(VI) from aqueous solution was conducted using *Rhizopus oryzae*, *Bacillus firmus*, and *Trichoderma viride* as microorganisms that can absorb Cr(VI). The research is focused on determination of optimum conditions including pH, the number of *R. oryzae*, *B. firmus*, and *T. viride* (inoculums), and initial concentrations of Cr(VI) used. Optimum pH was obtained at pH 5, 4.5 and 6, for biosorption of Cr(VI) with *R. oryzae*, *B. firmus*, and *T. viride*, respectively, in the capacity of 45.3%, 24.5%, and 90.3%. The highest amount of Cr(VI) adsorbed for biosorption with *R. oryzae*, *B. firmus*, and *T. viride*, were 55.4%, 18.5%, and 74.5%, respectively, using 6-mL inoculums. The equilibrium concentrations achieved for *R. oryzae*, *B. firmus*, and *T. viride* were 60 mg/mL, 40 mg/mL, and 40 mg/mL, with the amount of Cr(VI) adsorbed were 32.4%, 28.2%, and 89.3%, respectively. The adsorption capacity for *R. oryzae*, *B. firmus*, and *T. viride* were 45.3 mg/1x10⁶ colonies, 36.2 mg/1x10⁶ cells, and 77.8 mg/1x10⁶ colonies, respectively. Overall, the biosorbents effectivity order in the biosorption process of Cr(VI) are *T. viride* > *R. oryzae* > *B. firmus*.

1. Introduction

Heavy metal pollution caused by both natural processes and human activities is serious problem in the environment. The main consequences of heavy metal pollution is contamination of water system. At high concentrations, heavy metal ions poisons aquatic life in the waters. These metal ions are non-degradable and are persistent in the environment [1]. One of toxic metals ion is Cr(VI). Cr(VI) shows elevated toxicity, being related to nasal irritation and ulceration, chronic bronchitis and emphysema, cause liver and kidney damage, lung and skin cancer, in addition to damaging DNA by its highly genotoxic crosslink to DNA [2, 3]. Inhaling and/or ingesting Cr(VI) compounds has been shown to be cancerous due to its easy permeation of the cell membrane and elevated oxidizing potential, causing lung and stomach cancer [3]. Therefore, the removal or reduction of Cr(VI), in particular in the waters, is essential.

Heavy metals such as Cr(VI) have been removed from industrial effluents by the application of conventional metal removal techniques such as reverse osmosis, solvent extraction, ion exchange and chemical precipitation [4, 5]. These methods are encountered with certain major disadvantages such as high energy requirements, incomplete metal removal and generation of a large quantity of toxic waste sludge. Consequently, the search for alternative solutions has become of eminent importance, either alone or coupled with another conventional method already used to remove Cr(VI).



The potential for growth and adaptation of microorganisms when faced with adverse conditions and their versatility in biotechnological applications linked to modern biotechnology, has qualified microorganism biosorption agents known as biosorbents [6]. The use of biosorbents in the removal of Cr(VI) is promising when compared to the conventional techniques mentioned above.

In the current work, the biosorption of Cr(VI) in aqueous solution has been conducted using microorganisms, *R. oryzae*, *B. firmus*, and *T. viride*. The use of microorganism for adsorption process of metal ions have been conducted before in some studies [7-10]. To date, the microorganisms used for biosorption process are mostly bacteria [7, 10] and fungi [8, 9]. Results of these studies indicated that microorganisms had high adsorption capacity and high effectivity to adsorb the metal ions [7-10]. The proposed mechanism of action of the biosorption of metal ions by microorganism is by adsorption in the cell membranes or cell walls (reviewed in [11]). The positively charged ions of metals present in the solution are attracted to the cell and adsorbed on a negatively charged surface of the cell. In addition, the biosorption process of metal ions should consider a number physicochemical factors including pH, contact time, concentration, or temperature, when living microorganism are applied [12, 13]. Therefore, in this study, the biosorption of Cr(VI) using *R. oryzae*, *B. firmus*, and *T. viride* are conducted under the influences pH, the amount of biosorbents, and initial concentrations of Cr(VI) used.

2. Materials and Methods

2.1 Materials

Culture of *R. oryzae* and *B. firmus* were obtained from Department of Food Science and Technology, Brawijaya University, Malang, while culture of *T. viride* was obtained from the microbial stock collection of Food and Nutrition Laboratory, Gadjah Mada University, Yogyakarta. 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%), K₂Cr₂O₇, MgCl₂·2H₂O, KH₂PO₄, Na₂HPO₄, NaCl, NaKC₄O₆H₄, KCl, NH₄Cl, CH₃COOH (100%; $d = 1.05$ g/mL), NaCH₃COON, pepton, nutrient agar and Mueller Hinton Broth for microbiology. Water was purified using distillation technique. The growth media for all three species were potatoes dextrose agar (PDA). The determination of Cr(VI) concentrations 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 Methods

A loop full of *R. oryzae*, *B. firmus*, and *T. viride* was grown in the sterile PDA for 6 days at 37 °C. Next, bacteria and spores grown in the PDA was dissolved in 1 mL of sterile bi-distilled water. These were cultivated in 100 mL liquid growth medium in 250-mL Erlenmeyer flasks on a rotary shaker at room temperature, for 36 h at 150 rpm. The suspension was the microorganisms' inoculums.

Chromium(VI) solutions with concentration of Cr(VI) of 10 mg/mL were added into liquid medium, these were contacted with cultures of *R. oryzae*, *B. firmus*, and *T. viride*, separately. These mixtures were then incubated at room temperature for 24 h, with continuously shaken on an orbital shaker at 350 rpm. The mixture was filtered and the resulting supernatants were taken in 1 mL and placed in a beaker for the next steps. A solution of 0.1 M H₂SO₄ at pH 1 and 2 mL of 1,5-diphenylcarbazine were added to supernatants, and allowed to stand at room temperature for 5-10 min. The absorbance of the solutions was measured by UV-Vis spectrophotometer at a wavelength of 540 nm. Similar steps were repeated with variations in the pH: 4, 5.5, 5, 6, 6.5, and 7; in the number of inoculums: 2, 3, 5 and 6 mL; and in the concentrations of Cr(VI): 20, 40, 60, 80, and 100 mg/mL. At the end of each biosorption process, the filtrate was separated and the final Cr(VI) concentrations in the solution were determined using AAS spectrometer (AA-6200/Shimadzu), and Cr(VI) adsorbed (%) was calculated. The adsorption capacity was calculated in the optimum condition of pH, the number of inoculum, and concentration. The adsorption capacity (q) was calculated using the following formula:

$$q = \frac{(C_0 - C_t)V}{\text{adsorbent mass (the number of microorganisms)}}$$

where: C_o = initial concentration of Cr(VI) solution (mg/mL); C_t = concentration of Cr(VI) after biosorption process (mg/mL); and V = volume of solution (mL). 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 relationship between pH variations and the percentage of Cr(VI) adsorbed using three different microorganisms. The Cr(VI) uptake was highly affected by the initial pH of aqueous metal solution. In the range of pH 4–7, *R. oryzae*, *B. firmus*, and *T. viride*, showed slightly differences in their optimum pH in adsorbing Cr(VI). As shown in Figure 1, the optimum pH obtained for three species were different, and the biosorption capacity for Cr(VI) were also varied among those species. The highest biosorption capacity for Cr(VI) was obtained using *T. viride* at pH 6 with 90.3%, followed by *R. oryzae* at pH 5 with 45.3%, and *B. firmus* at pH 4.5 with 24.5%.

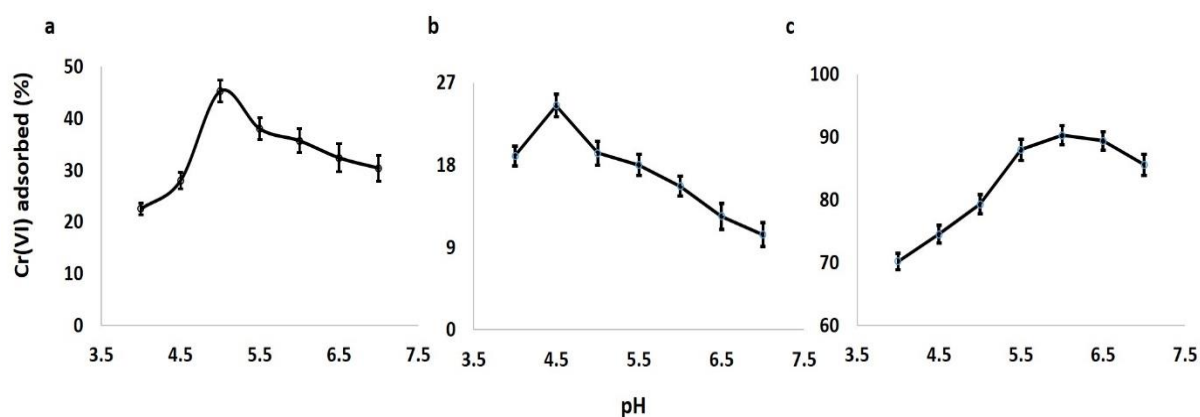


Figure 1. Result of biosorption of Cr(VI) using: (a) *R. oryzae*, (b) *B. firmus*, and (c) *T. viride*, under the influence of pH. The values are means and standard deviation of three replicates experiment.

The pH of the medium affects both the surface metal binding sites and metal chemistry in solution [11]. In solution, Cr(VI) exist in the form of its anions such as HCrO_4^- , CrO_4^{2-} , and $\text{Cr}_2\text{O}_7^{2-}$ [14]. Since the biosorption process was performed at acidic to neutral pH (4–7), these caused the H^+ ions in the solution were increased. As a result, Lewis base groups in the cell walls of fungi and bacteria, *i. e.* $-\text{NH}_2$ group was protonated to NH_3^+ , making it possible to interact with protonated Lewis base groups and forming ion pair complexes with Cr(VI) species. Several researchers have investigated the effect of pH on biosorption of Cr(VI) by using different kinds of microorganism and have reported identical results [15, 16].

In the current study, the differences in optimum pH and the variation in the amount of Cr(VI) adsorbed for *B. firmus*, *R. oryzae*, and *T. viride* indicates the nature of the species used influence the biosorption capacity of Cr(VI). The fungal cell walls as in the case for *R. oryzae* and *T. viride* are mainly composed of polysaccharides β -1,3 glucan and β -1,6 glucan, whereas that of the Gram-positive bacteria such as *B. firmus* is constituted of a thick layer composed of peptidoglycan [11]. It is shown in this study that the capacity of *R. oryzae* and *T. viride* were greater than *B. firmus* in adsorbing Cr(VI), under the influence of pH.

The percentage of Cr(VI) removal from aqueous solution was found to increase concomitantly with increments in the number of microorganisms as shown in Figure 2. These indicate that the binding sites on the cell walls of fungi or in the cell membranes of bacteria is also increased, as a result, biosorption of Cr(VI) increased. At the highest amount of inoculum used (6 mL) the percentage values of Cr(VI)

adsorbed by *B. firmus* was 18.5%, while *T. viride* showed the highest at 74.5%, and *R. oryzae* was at 55.4%. However, in these conditions, the adsorption capacity has not been achieved since the trend from all graphs (Figure 2a-2c) show that increasing inoculum will increase the amount of Cr(VI) adsorbed. Nonetheless, trend obtained in Figure 2 is in agreement with previous result, where the *T. viride* had the highest capacity in adsorbing Cr(VI), followed by *R. oryzae*, and *B. firmus*.

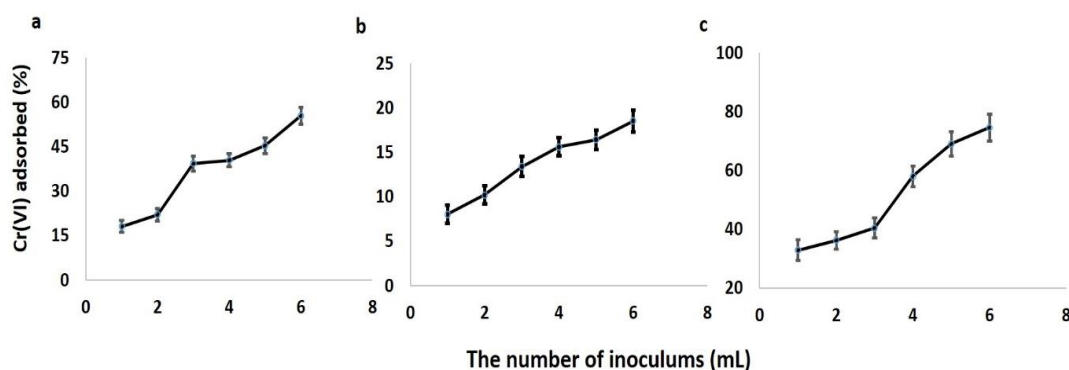


Figure 2. Result of biosorption of Cr(VI) using: (a) *R. oryzae*, (b) *B. firmus*, and (c) *T. viride*, under the influence of the number of inoculums (mL). The values are means and standard deviation of three replicates experiment.

The adsorption process of Cr(VI) is not only dependent on the availability of active sites for metal binding but also on the initial concentrations of Cr(VI). Effect of initial Cr(VI) ions concentration on its biosorption by *R. oryzae*, and *B. firmus*, and *T. viride* was investigated by incubating those species with previous optimum conditions that obtained before (pH 5, 4.5, or 6, for *R. oryzae*, and *B. firmus*, and *T. viride*, respectively, using 6-mL inoculum), with 100 mL of Cr(VI) solutions, concentration ranging from 20 to 100 mg/mL. Results are presented in Figure 3.

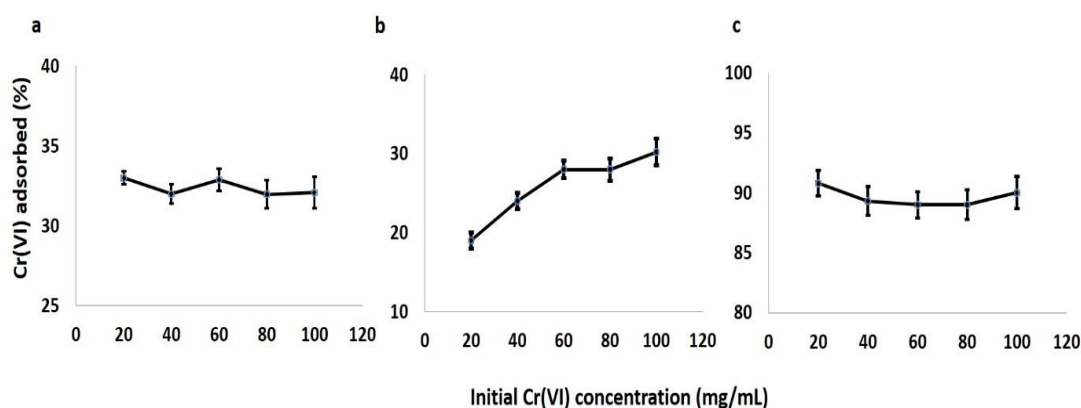


Figure 3. Result of biosorption of Cr(VI) using: (a) *R. oryzae*, (b) *B. firmus*, and (c) *T. viride*, under the influence of initial Cr(VI) concentration (mg/mL). The values are means and standard deviation of three replicates experiment.

From Figure 3, the uptake of Cr(VI) using *R. oryzae* and *T. viride*, showed similar trend at concentration of Cr(VI) ranging from 20-100 mg/mL. The equilibrium concentration obtained for *R. oryzae* and *T. viride* was at 40 mg/mL, in the percentages of 32.4% and 89.3%, respectively. For *B. firmus*, the concentration at equilibrium state achieved at concentration of 60 mg/mL, with 28.4% of

Cr(VI) adsorbed. The influence of concentration in the biosorption can be explained that at the beginning of the process, active sites at the cell walls of *T. viride* and *R. oryzae* or cell membrane of *B. firmus*, adsorbed metal ions fast. This may be due the unoccupied of the negatively active sites on the surface of cell walls, or may be due to the higher collision between the metal ions and negatively functional groups [14]. At high equilibrium concentration, uptake of Cr(VI) ions owing to surface binding was negligible due to saturation of biosorbent-binding sites. The slow increase in biosorption capacity at high concentrations could be related to the different concentration gradient between the solution and the inside of the microbial cells [15]. In these final conditions for three different biosorbent, the adsorption capacity for Cr(VI) was calculated. The calculated of the number of microorganisms for *R. oryzae*, *B. firmus*, and *T. viride* were 1.56×10^5 CFU/mL, 1.23×10^5 cells/mL, and 1.37×10^5 CFU/mL, respectively. Therefore, the adsorption capacity of *T. viride* toward Cr(VI) was 77.8 mg/1 x 10^6 colonies *R. oryzae* was 45.3 mg/1x 10^6 colonies, and *B. firmus* was 36.2 mg/1x 10^6 cells.

Again, the biosorption result of Cr(VI) under the influence of concentration show that *T. viride* had the highest capacity to adsorb Cr(VI), followed by *R. oryzae*, while *B. firmus* had the lowest capacity. Metal uptake mechanisms by various biosorbents depend on the cellular surface of the microbes. Generally, all microorganisms have a negative charge on their cell surface due to presence of anionic structures, that enable them to bind to metal ions. These negative charged groups includes alcohol, amine, carboxyl, ester, phosphoryl, sulfonate, sulfhydryl, and thiol group [17]. However, analysis of cell wall component, which also vary among different microbes, helps in assessing metal uptake by different microorganisms. In Gram-positive bacteria, such as *B. firmus*, peptidoglycan layer, which contains alanine, glutamic acid, polymer of glycerol and teichoic acid are the active sites involved in metal binding process [11]. Metals are attached to these ligands on cell surfaces, which displace essential metals from their binding sites [18].

The rigid cell walls of fungi, such as *R. oryzae* and *T. viride*, consist of chitin, inorganic ions, lipids, nitrogen-containing polysaccharide, polyphosphates, and proteins [19]. The surface of their cell wall acts as a ligand for binding metal ions, resulting in the removal of metals. Furthermore, fungi can also absorb heavy metals into their mycelium and spores [19, 20]. Therefore, it is suggested that *R. oryzae* and *T. viride* had higher capacity in adsorbing Cr(VI) compared to *B. firmus*, due to the mycelium and spores also contributed to biosorption process of Cr(VI). The differences between *R. oryzae* and *T. viride* show that microbial has different biosorptive abilities and effectivities, which also varies considerably within each group and each species.

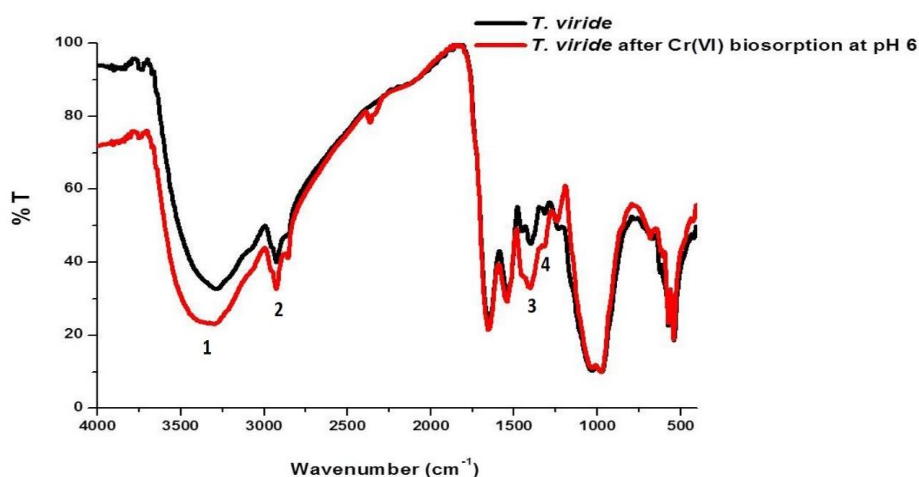


Figure 4. The FTIR spectra from *T. viride* and after biosorption process of Cr(VI) by *T. viride* at pH 6.

The functional groups in the biosorbents contributed to biosorption process of Cr(VI) separated from their filtrates and have been characterized using FTIR spectrophotometry. Figure 4 shows the FTIR

results from *T. viride* before and after biosorption process in the pH 6. The *T. viride* was chosen to represent the biosorbent in Cr(VI) uptake, since it has the highest adsorption capacity among three different biosorbents. Some changes in FTIR bands were observed before and after biosorption process. The changes and relative intensities in the FTIR peaks are tabulated in Table 1. As shown in Figure 4, there were four significant areas affected due to biosorption process of Cr(VI). Those areas were 3450-3100 cm^{-1} , 2950-2850 cm^{-1} , 1470-1450 cm^{-1} , and 1180-1160 cm^{-1} . The band assignment for those are N-H bending from amine group, C-H stretching from alkane, another C-H stretching from alkane, and C-N bending from amine group, respectively [21, 22]. In addition, in the finger printing regions (1000-500 cm^{-1}) also showed some changes, these regions are generally phosphate groups and alkanes bands from nucleic acids (DNA). All those bands showed decreases in intensity relative to *T. viride* bands in the same regions. This is possible since *T. viride* as biosorbent is also microorganisms, that contains biomolecules, including proteins, lipids, carbohydrates, and nucleic acids.

Table 1. FTIR changes in intensity of bands of interests relative to *T. viride* bands after biosorption of Cr(VI) at pH 6

No	Wavenumber (cm^{-1})	Assignment	Intensity changes*
1	3450-3100	N-H amine	↓
2	2950-2850	C-H alkane	↓
3	1470-1450	C-H alkane	↓
4	1180-1160	N-H amine	↓

*The changes are relative compared to the intensity in the related FTIR bands of *T. viride*

4. Conclusion

The biosorption process of Cr(VI) by three different microorganisms, *R. oryzae*, *B. firmus*, and *T. viride* has been conducted successfully. The optimum pH obtained for *R. oryzae*, *B. firmus*, and *T. viride* were 5, 4.5 and 6 with the Cr(VI) adsorbed 45.3%, 24.5%, and 90.3%, respectively. Using 6-mL inoculum, the biosorption capacity for Cr(VI) were 55.4%, 18.5%, and 74.5%, with *R. oryzae*, *B. firmus*, and *T. viride*, respectively. The concentration in the equilibrium state for *B. firmus* and *T. viride* achieved at 40 mg/mL with Cr(VI) adsorbed were 28.2% and 89.3%, respectively, while *R. oryzae* was at 60 mg/mL (32.4%). Therefore, the order in the biosorption process of Cr(VI) using microorganisms as adsorbents are *T. viride* > *B. firmus* > *R. oryzae*, with adsorption capacity of 77.8 mg/1x10⁶ colonies > 45.3 mg/1x10⁶ cells > 36.2 mg/1x10⁶ colonies, respectively.

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