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COBALT (II) BIOSORPTION USING TRICHODERMA VIRIDE IMMOBILIZED IN CALCIUM ALGINATE

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ABSTRACT

Cobalt is one of the heavy metals found in water and comes from the wastes of petroleum, paint, glass and ceramics industries. High concentrations of cobalt can cause paralysis, diarrhea, low blood pressure, lung irritation and bone defects. Biosorption is a method that can be used to reduce the concentration of Co(II) ions in water using microorganisms. The research focused on the determination of optimum conditions of pH, contact time, steering speed, and the amount of adsorbent on biosorption of Co(II) by Ca-alginate immobilized *Trichoderma viride*. Co(II) solution was adsorbed at pH 1-6 for 1-8 hours and a steering speed of 50-150 rpm with 0.25-2 grams of adsorbent. The analytical method used for determining Co(II) concentration is visible light spectrophotometry using Nitroso-R salt reagent. The results showed that the optimum biosorption occurred at pH 4, with a contact time of 4 hours, stirring speed of 100 rpm, and 1.5 grams of adsorbent with the amount of Co(II) adsorbed of $(55.37 \pm 0.32)\%$ and adsorption capacity of Co(II) is 0.1496 mg/g adsorbent.

KEY WORDS

Biosorption, cobalt, immobilized, *Trichoderma viride*.

Water pollution is a serious environmental problem. One of the causing factors is the presence of heavy metals in aquatic ecosystems. Heavy metal elements are generally toxic and tend to form ions in water [1]. Several types of heavy metals often found in polluted water bodies include Hg, Pb, Cd, Mn, Ag, Fe, Co, Ni, Cu, Cr, Zn. The toxic power of heavy metals functions as a barrier to enzyme work that can break the body's metabolic process. Furthermore, these heavy metals act as allergens, mutagens, teratogens or carcinogens for humans [2]. Cobalt is an element whose presence in waters comes from the wastes of petroleum, paint, glass, and ceramic industries. According to the Environmental Protection Agency, the maximum limit of [Co] in drinking water is 0.2 mg/ L. Cobalt concentrations exceeding the threshold can cause paralysis, diarrhea, low blood pressure, lung irritation and bone defects [3].

Biosorption has a high potential contribution to reducing heavy metal content even at low concentration levels [4]. Several algae, bacteria, fungi, and yeasts have been proven to be able to adsorb metals [5]. One type of fungus that can be used as an adsorbent is *Trichoderma viride*. *T. viride* has compounds with functional groups that are capable of binding metal cations such as carbonyl, hydroxyl, carboxyl, phosphate, and amino [6].

This research applied immobilization so that adsorbent can be used continuously and repeatedly [8]. Alginate is one of the immobilizer matrices due to its suitability for immobilizing all types of cells, such as bacteria, yeast, fungi, plant cells, and animal cells as well as its capability to maintain the biocatalyst process optimally [9]. Alginate also has the adsorptive properties contained in the carboxylate and hydroxyl groups, known to have a high affinity for heavy metals [10].

METHODS OF RESEARCH

The equipment used in this research was a set of glassware, a suction ball, a test tube rack, a spray bottle, an analytical balance, a shaker, inoculating loops, cotton, sterile gauze,



brown paper, laminar air flow, Bunsen, autoclave, incubator, incubator, Spectronic 20, FTIR, refrigerator, stirrer, magnetic stirrer, and pH meter.

The materials used in this research were samples of *Trichoderma viride*, distilled water, potato, dextrose, agar, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, nitroso-R Salt, glacial acetic acid, sodium acetate, peptone, oleic acid, sodium alginate, KH_2PO_4 , $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $(\text{NH}_4)_2\text{SO}_4$, and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.

Preparation of 100.0 ppm Co (II) Stock Solution. $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ solid was weighed as much as 0.1011 g and then put into a beaker. The solid was dissolved using distilled water. The solution was then transferred to a 250 mL volumetric flask with the addition of distilled water up to the border mark and then shaken until homogeneous.

Preparation of Co(II) Standard Solution. Standard Co(II) solution of 2.0; 4.0; 6.0; 8.0; and 10.0 ppm was made from 100 ppm Co(II) stock solution. The 100-ppm stock solution was then pipetted, each of which amounted to 2.0; 4.0; 6.0; 8.0; and 10.0 mL to be then put into a 100 mL volumetric flask. Lastly, distilled water was added to this solution up to the border mark and then shaken until homogeneous.

Determination of Co(II) Maximum Wavelength. Co (II) solution of 6.0 ppm was pipetted as much as 10.0 mL and then put into a 25 mL volumetric flask. Furthermore, the pH of the solution was measured to 5.5 and then a total of 2.0 mL of 0.2% nitroso-R salt was added. After that, acetate buffer pH 5.5 was added to this solution up to the border mark and shaken until homogeneous. The absorbance was measured by Spectronic 20 at a wavelength of 400 – 600 nm.

Preparation of Co(II) Standard Curve. Standard Co(II) solution of 2.0; 4.0; 6.0; 8.0; and 10.0 ppm was pipetted as much as 10 mL each into a 25 mL volumetric flask. Furthermore, the solution pH was measured to 5.5 and then a total of 2.0 mL of 0.2% nitroso-R salt was added. After that, acetate buffer pH 5.5 was added up to the border mark and shaken until homogeneous. The absorbance was measured by Spectronic 20 at the maximum wavelength obtained.

Preparation of T. viride Fungus Biomass. Fungi cultured on PDA (Potato Dextrose Agar) were put into a liquid medium containing peptone, KH_2PO_4 , $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $(\text{NH}_4)_2\text{SO}_4$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, oleic acid, dan acetate buffer pH 5 in Erlenmeyer. It was then dissolved in 250 mL of sterile distilled water and shaken using a shaker for 36 hours at a speed of 150 rpm.

Immobilization of T.viride in Ca-alginate. Sodium alginate of 1.25 g was added to the *T.viride* biomass while being heated at a temperature of 40° C and stirred until dissolved. Moreover, CaCl_2 solution with a concentration of 3% (w/v) was prepared and heated at a temperature of 60° C. The *T. viride* biomass mixed with sodium alginate was dropped into a 3% CaCl_2 using a dropping pipet while stirring with a stirrer [11].

Determination of the Effect of pH on Co(II) Biosorption. 20.0 ppm Co(II) solution of 20.0 mL with various pH (1, 2, 3, 4, 5, and 6) was put into an Erlenmeyer. It was then added with immobilized *T. viride* of 0.5 grams and shaken using a shaker for 6 hours. After that, the Co(II) solution was filtered. The absorbance of the obtained filtrate was then measured by Spectronic 20.

Determination of the Effect of Contact Time on Co(II) Biosorption. 20.0 ppm Co(II) solution of 20.0 mL at the optimum pH was put into an Erlenmeyer. It was then added with immobilized *T. viride* of 0.5 grams and shaken using a shaker with time variations of 1, 2, 4, 6, and 8 hours. After that, the Co(II) solution was filtered. The absorbance of the obtained filtrate was then measured by Spectronic 20.

Determination of the Effect of Stirring Speed on Co(II) Biosorption. 20.0 ppm Co(II) solution of 20.0 mL at the optimum pH was put into an Erlenmeyer. It was then added with immobilized *T. viride* of 0.5 grams and shaken using a shaker with the optimum contact time and stirring speed variations of 50; 75; 100; 120; and 150 rpm. After that, the Co(II) solution was filtered. The absorbance of the obtained filtrate was then measured by Spectronic 20.

Determination of the Effect of Adsorbent Amount on Co(II) Biosorption. 20.0 ppm Co(II) solution of 20.0 mL at the optimum pH was put into an Erlenmeyer. It was then added with immobilized *T. viride* with mass variations of 0.25; 0.5; 0.75; 1.0; 1.25; 1.5; 1.75; dan 2.0



grams and shaken with the optimum contact time and stirring speed. After that, the absorbance of the obtained filtrate was then measured by Spectronic 20.

Determination of Immobilized T.viride Functional Groups Using FTIR. Immobilized T.viride in Ca-alginate before and after being contacted with Co(II) solution was analyzed using an FTIR spectrophotometer. Identification was conducted by firing infrared rays into the plate containing the dried sample to produce spectra with a certain intensity at a wave number of 400 - 4,000 cm^{-1} [12].

RESULTS AND DISCUSSION

The Effect of pH. pH is one of the factors greatly affecting the biosorption process. It's due to pH affects the surface charge of sorbent by protonation of functional group in the biomass [13]. The solution pH affects the solubility of metal ions and the activities of adsorbent functional groups. At low pH, the surface of the adsorbent is protonated to inhibit the biosorption process. Meanwhile, at high pH, metals tend to precipitate.

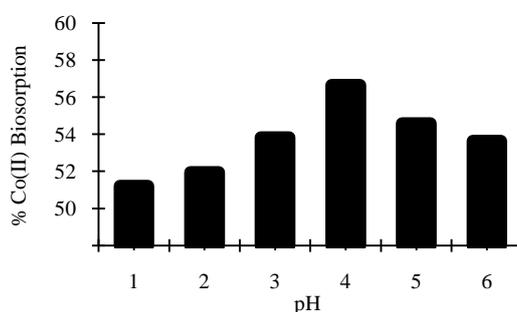


Figure 1 – The Curve of the Effect of pH on Co(II) Biosorption

Based on the research results, the percentage of Co(II) biosorption increased from pH 1 to pH 4 and then decreased to pH 6. The increase in biosorption percentage was caused by the presence of H^+ ions in the solution that could compete with Co(II) for binding to the adsorbent functional groups. The decrease in biosorption percentage was caused by the hydrolysis of Co(II) metals that eventually weakened the interaction between Co(II) and the adsorbent.

The Effect of Contact Time. The optimum contact time is determined to obtain the highest metal ion adsorption power. The percentage of metal ion biosorption increases because the surface area of the adsorbent available for metal biosorption is large.

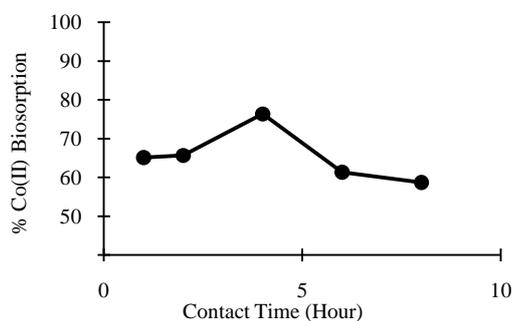


Figure 2 – The Curve of the Effect of Contact Time on Co(II) Biosorption

The interaction between Co(II) and the adsorbent from the beginning to the 4th hour reflected the increase in the percentage of Co(II) biosorption. However, in the next hours, the percentage of Co(II) biosorption decreased. The longer contact time leads to a more amount of Co(II) bound to the adsorbent functional groups until it reaches equilibrium. The percentage of Co(II) biosorption can decrease because not all bonds occurring between



Co(II) and the adsorbent are electrostatic bonds. Therefore, at a long enough contact time, desorption can occur on the saturated adsorbent.

The Effect of Stirring Speed. The stirring speed affects the contact between adsorbents and adsorbates.

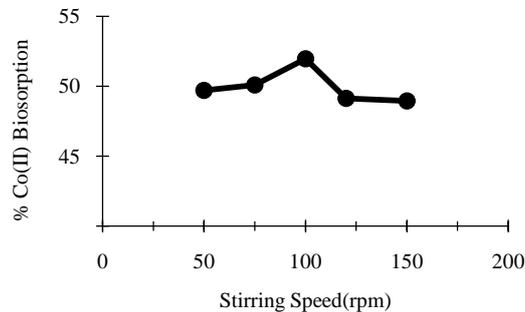


Figure 3 – The Curve of the Effect of Stirring Speed on Co(II) Biosorption

The faster the stirring speed, the higher the adsorption rate. More opportunities for contact between Co(II) and adsorbents can cause Co(II) to increasingly bind to the adsorbent. The optimum biosorption was achieved at a stirring speed of 100 rpm. Too high stirring speeds lead to releasing of the bond between adsorbents and adsorbates, eventually decreasing the percentage of Co(II) biosorption.

The Effect of Adsorbent Mass. Adsorbents act as a provider of functional groups to be able to bind to metal ions.

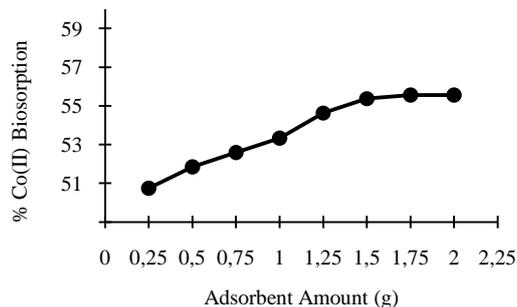


Figure 4 – The Curve of the Effect of Adsorbent Mass on Co(II) Biosorption

The percentage of Co(II) biosorption got higher along with the increasing mass of adsorbents until the total adsorbent of 1.5 g, then tended to be constant. A greater amount of adsorbent leads to an increased number of active groups available to bind with Co(II) up to optimum conditions. The amount of adsorbent above the optimum conditions will not affect the biosorption process. This is because the greater amount of adsorbent will lead to more contact between the adsorbent and adsorbate.

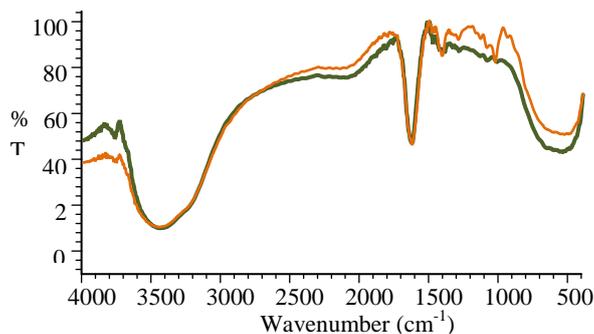


Figure 5 – FTIR Spectra of Immobilized *T. viride* before (—) and after (—) being contacted with Co(II)



Functional Group Analysis of Immobilized T. viride using FTIR. Figure 5 depicts a widening adsorption peak at a wavenumber of 3421.48 cm^{-1} , indicating the presence of a carboxylate O-H group. At a wavenumber of 1635.52 cm^{-1} , there is strong adsorption indicating the presence of a C=O group. Carboxylic and hydroxyl groups are functional groups making up Ca-alginate and *T. viride* fungi [1, 6].

The Co-O bond formed between Co(II) and adsorbent functional groups is an ion pair bond, so it cannot adsorb the IR wavenumber area. The interaction between Co(II) and adsorbents can be seen from the change in adsorption intensity at a wavelength of $500\text{-}600\text{ cm}^{-1}$. In other words, the presence of Co(II) can lower the value of the dipole moment and adsorption intensity

Adsorption Capacity. Adsorption capacity is the amount of adsorbate taken up by the adsorbent per unit mass (or volume) of the adsorbent. Adsorption capacity is calculated based on the optimum conditions obtained from 4 biosorption factors that have been studied. By using equation [1]:

$$q = \frac{(C_0 - C_t)V}{w} \quad (1)$$

This research obtained an adsorption capacity of 0.1496 mg/g .

CONCLUSION

pH, contact time, stirring speed, and amount of adsorbent greatly affect the biosorption of Co(II) using immobilized *T. viride* in Ca-alginate. The optimum conditions were reached at pH 4 with a contact time of 4 hours, stirring speed of 100 rpm, and adsorbent amount of 1.25 g. The adsorption capacity obtained was 0.1496 mg/g adsorbent.

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