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Physico-Chemical Characteristics of Biofilm to Display the Rate of Cr Adsorption in the Manyar Gresik River

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ABSTRACT

Chromium (Cr) pollution caused by industrial activities can have a negative impact on the balance of aquatic ecosystems, aquatic life, and human health. Therefore, effective, cheap, and environmentally friendly handling efforts are needed to reduce chromium levels in the aquatic environment. One potential approach is the use of natural biofilm-based biosorbents. Biofilm has advantageous characteristics, including its abundant availability in nature, high heavy metal absorption ability, and its stability in a wide range of aquatic environmental conditions. This study uses biofilms that grow naturally on the surface of rocks in the Manyar River flow, Gresik, Indonesia, which is known as one of the areas with fairly high industrial activity. The research method is experimental, utilizing two main indicators, namely adsorption kinetics and adsorption isotherms. Kinetic parameters are used to determine the rate of absorption of chromium by biofilms over a given time span, while isothermal models are used to understand the mechanism of interaction between chromium metal ions and biofilm surfaces. The results of the study show that the process of kinetic adsorption by biofilm takes place very quickly. This is indicated by an adsorption capacity value of 4.405 – 4.390 mg/g, with the optimal contact time reached at 5 minutes. Meanwhile, isothermal analysis of adsorption indicated that the adsorption mechanism followed the Langmuir model. This is supported by a determination coefficient value (R^2) of 0.952 and a maximum capacity value (Nmax) of 56.50 mg/g, which indicates that the adsorption process occurs in a single layer (monolayer) on the surface of the biofilm. These findings confirm that natural biofilms derived from Kali Manyar rocks have great potential as effective and sustainable biosorbents, especially in efforts to reduce levels of chromium metal in polluted aquatic environments.

1. INTRODUCTION

Chromium is one of the heavy metals that are widely reported in various kinds of literature as an important pollutant component in the environment, in soil, air, and water. These elements naturally come from parent rocks and minerals in the earth's crust, which are released into the environment due to geological weathering processes [1]. Under natural conditions, the chromium content in the environment is usually low and relatively stable. However, changes in ecosystem conditions and human intervention can convert its existence to a more reactive and dangerous form. Chromium is known to be highly toxic and easily soluble in water, so it has high mobility and significant bioaccumulation potential [2].

Apart from natural sources, chromium can also originate as a result of various anthropogenic activities. The main sources of this metal contamination are the tanning industry, electroplating, metallurgy, stainless steel production, as well as the use of chemicals in agriculture, such as certain pesticides and fertilizers [3]. The increasingly rapid urbanization and industrialization processes have contributed greatly to the increase in heavy metal levels in the waters. Industrial liquid waste that is not treated properly usually contains chromium in high concentrations and can contaminate water bodies. The type and level of incoming contaminants are highly dependent on the waste treatment system applied, so countries or regions with inadequate waste management tend to have high levels of heavy metal pollution [4].

One of the approaches that have been widely developed is the use of biosorbents, which are biological materials that have the ability to absorb heavy metal ions from wastewater. The advantages of biosorbents lie in their relatively low cost, their abundant availability in nature, as well as their ability to be reused after the desorption process [5]. The ideal biosorbent should have high adsorption capacity, good chemical stability, and regenerative ability without loss of efficiency. Some potential sources of biosorbents include agricultural waste, microbial biomass, and biofilm, which show high performance in absorbing heavy metals from industrial wastewater [6].

Biofilm is one of the forms of natural biosorbents that are very interesting to study. Biofilms are formed by communities of microorganisms attached to the surface and surrounded by a matrix of extracellular polymeric substances (EPS). The main components of biofilms include water, living and dead microbial cells, EPS, and extracellular DNA (eDNA), which interact with each other to form complex three-dimensional structures. These structures create waterways and microspaces that allow for the exchange of gases and nutrients, making the biofilm a dynamic and living system. EPS itself serves to protect microbial cells and provides an active site for metal ion binding [7].

The EPS component of biofilm contains anionic groups such as carboxylates, hydroxyls, and phosphates that have the ability to electrostatically bind heavy metal ions [8]. In addition, the interaction between eDNA and polysaccharides also strengthens the binding mechanism through ion exchange. These active groups play an important role in the adsorption process because they are able to attract metal cations from the solution and form complex bonds on the surface of the biofilm. This mechanism depends not only on the presence of functional groups but also on the pH of the solution, the concentration of metal ions, and the morphology of the biofilm itself. Thus, biofilms have unique abilities that are adaptive to the surrounding environmental conditions [9].

Kinetically, the process of adsorption of heavy metals by biofilms is rapid because it involves physico-chemical mechanisms [10]. In many cases, adsorption occurs through a combination of ion exchange and electrostatic interactions between the surface of the negatively charged biofilm and the positively charged ions of the heavy metal [11]. This adsorption is a passive process, meaning it does not require direct microbial metabolism, but occurs due to the chemical attraction between the charge and the surface interaction. When the entire active site on the biofilm has been filled with metal ions, the adsorption process reaches an equilibrium point, which signifies the material's maximum capacity to handle the metal [12].

In this study, the authors focused on examining the adsorption characteristics of biofilms against chromium (Cr) heavy metals using adsorption kinetics (second-order pseudo-order) and isothermal adsorption approaches (Langmuir model). Through kinetic tests, it can be determined the rate of absorption of metal ions by the biofilm as well as the dominant type of mechanism. Meanwhile, isothermal models provide an understanding of the properties and maximum adsorption capacity of biofilm surfaces. The results show that biofilm is able to absorb Cr metal quickly and efficiently without going through biologically active processes. This process occurs passively, driven by electrostatic interactions and ion exchange between the functional groups of the biofilm and the Cr ions within the solution.

However, until now, research on the use of natural biofilms as biosorbents in the heavy metal adsorption process is still relatively limited. Most previous research has focused more on the use of pure microbial biomass or engineered biofilms under controlled laboratory conditions. As a result, there are still research gaps related to the understanding of adsorption mechanisms, adsorption capacity, and stability of natural biofilms that form spontaneously in the real environment. Further study on this is important to fill the gap.

2. MATERIALS AND METHODS

2.1 Research location

This research was carried out in December 2023 at the Halal Center Laboratory, State Islamic University (UIN) Malang, East Java, Indonesia. This research activity includes the stage of sampling biofilm that grows naturally in the Manyar River basin, Gresik, which was chosen as the research location because of its environmental conditions that support the formation of natural biofilm on various aquatic substrates and large-scale industrial activities, such as the production of paints and metal coatings using chromium compounds in their production, to produce waste that can pollute these waters. The collected biofilm samples were then taken to the laboratory for further analysis regarding their adsorption characteristics to obtain a comprehensive picture of the potential of the biofilm in the context of adherence to chromium heavy metals.

2.2 Biosorbent extraction of biofilm

The biofilm sampling procedure was initiated by carefully separating the biofilm from the surrounding sediment and any attached macro-organisms. This step was performed using sterile tweezers to maintain sample integrity and minimize potential contamination. Such preliminary handling was critical to ensure that the collected biofilm accurately represented the in-situ condition prior to further processing. Before the brushing stage, a plastic container filled with 80 mL of distilled water (Aquadest), along with its lid and a toothbrush, was weighed to obtain the initial mass. The biofilm, still attached to the stone substrate, was subsequently brushed in a consistent direction into the container. This process was conducted gently to ensure complete detachment of the biofilm while preserving its structural components necessary for subsequent analyses [13].

After collection, the container holding the biofilm suspension, together with the lid and toothbrush, was reweighed to determine the final mass. The difference between the initial and final weights corresponded to the wet biomass of the streamer biofilm, which was approximately 0.8 g. The biofilm suspension was then transferred into a pre-cleaned, labeled film bottle and sealed with plastic wrap to prevent contamination and evaporation. Finally, the samples were stored in a cool box at approximately 4 ℃ to maintain biofilm stability and to minimize alterations in microbial activity prior to further analysis [14].

2.3 Kinetics of adsorption

At this stage, 50 mL of $K_2Cr_2O_7$ solution was incubated with 0.8 g of biofilm pellets at 28 ℃ and a stirring speed of 125 rpm to ensure homogeneous interaction between the adsorbate and the adsorbent. Contact times of 5, 15, 30, and 60 minutes were applied to evaluate the adsorption rate and the attainment of equilibrium conditions. The adsorption kinetics were analyzed using a pseudo-second-order model.

After incubation, the liquid and solid phases were separated by centrifugation at 6,500 rpm for 30 minutes. The residual chromium concentration in the supernatant was then measured using an Atomic Absorption Spectrophotometer (AAS, Shimadzu AA-6800).

The adsorption capacity (q_t, mg g^{-1}) at time t was calculated using the following equation:

$$q_t = \frac{(C_0 - C_t) \times V}{m}$$

where,

q_t = adsorption capacity at time t (mg g^{-1});
C_0 = initial chromium concentration (mg L^{-1});
C_t = chromium concentration at time t (mg L^{-1});
V = volume of solution (L);
m = dry weight of biofilm (g).

The dry weight of the biofilm was determined prior to the adsorption experiment to ensure accurate normalization of adsorption capacity. Biofilm pellets were first collected and washed with distilled water to remove residual medium and loosely attached particles. The samples were then oven-dried at 60 °C until a constant weight was achieved (typically 24–48 hours). The dried biofilm mass was measured using an analytical balance and recorded as the dry weight (m) used in the adsorption calculations [14, 15]. To ensure data accuracy, all experiments were conducted in triplicate.

2.4 Isotherm adsorption

This stage was used to determine the adsorption capacity of the biofilm against heavy metals as well as to assess the suitability of the data with the Langmuir isothermal model, which assumes that adsorption occurs on a homogeneous surface with a limited number of sites with no interaction between adsorbate molecules. The experiment was carried out by preparing a solution of K_2CrO_7 at various initial concentrations (10-1000 mg/L), each as much as 80 mL, then 0.8 grams of biofilm pellets were added and stirred with a magnetic stirrer at 125 rpm for 5 minutes. The pH condition was maintained around 7 at 28 °C to maintain the stability of the system and prevent chromium precipitation. Afterwards, the mixture is decentralized at 6,500 rpm for 10 minutes, and the supernatant was measured for chromium content with AAS (Shimadzu AA-6800).

Chromium biosorption by biofilm was analyzed using variants of the Langmuir equation as described by Kurniawan et al. [14].

$$\frac{C}{N} = \frac{1}{(N_{max})b} + \frac{C}{N_{max}} \tag{1}$$

where,

C = equilibrium concentration of chromium ions in solution (mg/L)

N = amount of chromium adsorbed per unit mass of adsorbent at equilibrium (mg/g)

N_{max} = maximum monolayer adsorption capacity of the adsorbent (mg/g)

B = Langmuir constant related to the adsorption affinity or the ratio of adsorption to desorption rates (L/mg)

This equation illustrates the dynamic equilibrium between the chromium ions in the solution and the adsorbed ions N at equilibrium concentration C, with the Langmuir constant b showing the ratio between the adsorption and desorption rates. The value of b increases as the adsorption rate dominates compared to desorption. In mathematical analysis, the mapping of the relationship between C/N and C produces a straight line with a slope of $1/N_{max}$ and a boundary point on the y-intercept $1/(N_{max})b$. From the graph, the values of N_{max} and b can be estimated, so that the interaction characteristics of chromium ions with the surface of the biofilm can be explained quantitatively.

2.5 Data analysis

The processing of experimental data was carried out through descriptive statistical analysis to trace the pattern of relationships between variables in the study. This approach provided a preliminary overview of the data trends, their distribution, and the general characteristics of the measurement results. Each graph produced contained an average value along with a standard deviation, so that the variation and consistency of the data can be clearly visualized. This presentation not only helped in understanding the behavior of the data but also served as the basis for the interpretation of the stability of the experimental results [16].

The next stage involved the application of linear regression analysis to test the relationship between variables that are directly related, such as the ratio of C/N to concentration C in the Langmuir model. Through this analysis, the direction and strength of the relationship could be determined, showing the extent to which chromium concentrations affect the amount of metals adsorbed by the biofilm. All analyses, both descriptive and regressive, were carried out using Microsoft Excel because of its ease in processing laboratory data and its ability to produce accurate results efficiently [17].

3. RESULTS AND DISCUSSIONS

3.1 Kinetic adsorption

Observation of contact time in the adsorption of heavy chromium metals was carried out at several time intervals, namely 5, 15, 30, and 60 minutes, with a chromium solution concentration of 50 mg/L. The results showed that the value of chromium heavy metal content at 5 min was recorded at 4.405 mg/g, at 15 min at 4.400 mg/g, at 30 min at 4.406 mg/g, and at 60 min at 4.390 mg/g. To provide a clearer picture of the relationship between contact time and chromium uptake, the results of these observations are presented visually in the following Figure 1.

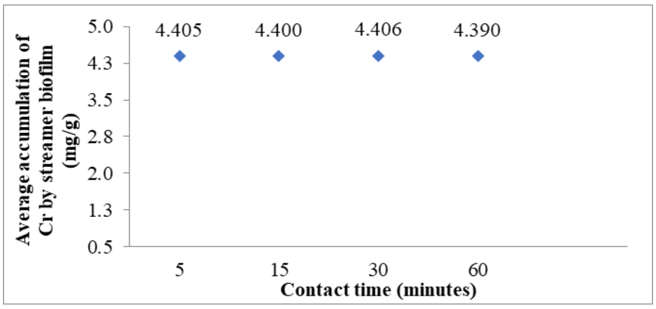


Figure 1. Contact time of biofilm with Cr

In the time range of 5 to 60 minutes, the accumulation of Cr in biofilm per gram of biofilm weight tends to be stable. This shows that the adsorption process takes place very quickly, namely at a contact time of 5 minutes. Based on the graph above, the best contact time is found at 5 min, which is relatively very short. Meanwhile, to see the equilibrium condition, it is necessary to give a difference in concentration and observed in isothermal adsorption with a contact time of 5 minutes.

The rapid adsorption process shows that the process of adsorption of Cr by biofilm takes place physico-chemically. The physico-chemical adsorption process is an adsorption process without going through metabolism and occurs very quickly. This follows the study [15], which states that biofilm matrices can be biological agents in monitoring water conditions due to exposure to heavy metals through physico-chemical interactions.

The main component of the biofilm matrix is interstitial water at 97%, which has a role in distributing nutrients. The biofilm layer has a thickness of approximately 10 to 30 mm with an EPS matrix layer thickness of about 0.2 to 1.0 mm. EPS makes up the majority of biofilms (65–95%) [18].

EPS, often referred to as biofilm polymers, are thought to contain charged sites in the biofilm matrix [19]. Biofilms have the ability to rapidly absorb heavy metal ions through a matrix of negatively charged EPS [20]. Biofilm polymers carry both negatively charged and positively charged sites due to ionization of functional groups, such as carboxyl and amino groups, in biofilm polymers [21].

The interaction of active functional groups on the surface of microbial cells and metal ions aids in the adsorption of heavy metals [22]. Physical adsorption is primarily dependent on the van der Waals force, while active or chemical adsorption involves the interaction between the absorbent and the adsorbate. The main attraction for adsorption is the electrostatic interaction between charged and uncharged compounds. To support the adsorption of heavy metals on microbial surfaces, this combination of mechanisms and interactions functions either simultaneously or separately [23].

3.2 Isotherm adsorption

The isothermal adsorption results for chromium (Cr) demonstrated a clear variation in equilibrium concentration, adsorption capacity, and removal efficiency across different initial concentrations. At an initial concentration of 10 mg L^{-1}, the equilibrium concentration (C_e) was 4.151 mg L^{-1}, corresponding to an adsorption capacity of 0.585 mg g^{-1} and a removal efficiency of 57.7%. Increasing the concentration to 30 mg L^{-1} resulted in a C_e of 12.792 mg L^{-1}, with q_e reaching 1.721 mg g^{-1} and efficiency decreasing to 49.1%. At 62.5 mg L^{-1}, the equilibrium concentration increased to 33.065 mg L^{-1}, accompanied by an adsorption capacity of 2.943 mg g^{-1} and an efficiency of 46.1%.

At higher initial concentrations, a similar trend was observed. For 250 mg L^{-1}, the equilibrium concentration reached 152.548 mg L^{-1}, with an adsorption capacity of 9.745 mg g^{-1} and efficiency of 37.8%. At 500 mg L^{-1}, the C_e increased to 316.326 mg L^{-1}, while adsorption capacity and efficiency were 18.367 mg g^{-1} and 36.6%, respectively. Further increases to 750 mg L^{-1} resulted in a C_e of 517.785 mg L^{-1}, with adsorption capacity of 23.222 mg g^{-1} and efficiency declining to 30.9%. At the highest concentration tested (1000 mg L^{-1}), the equilibrium concentration reached 731.664 mg L^{-1}, with the highest observed adsorption capacity of 26.834 mg g^{-1}, although the removal efficiency decreased to 26.6%.

To further evaluate the adsorption behavior, the relationship between chromium accumulation in the biofilm and the equilibrium concentration is illustrated in Figure 2, and relationship between initial concentration and removal efficiency in Figure 3.

Adsorption of Cr by biofilms at high concentrations shows more constant values than at lower concentrations. This is because the active sites of the biofilm are fully filled with Cr ions, so that the addition of concentrations in water will not increase the amount of Cr adsorption by the biofilm [24].

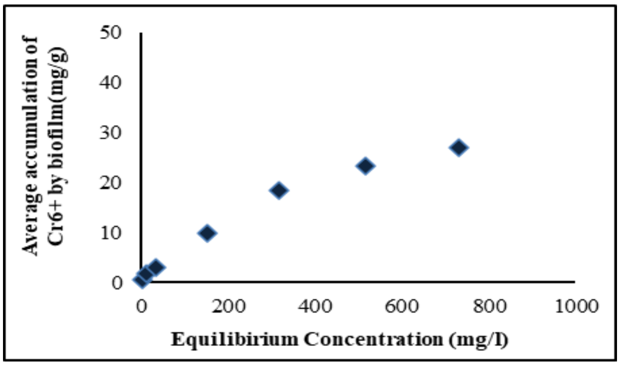


Figure 2. Distribution of accumulated Cr

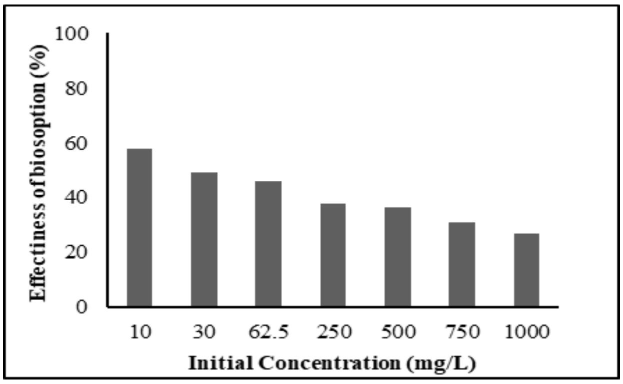


Figure 3. Efficiency of chromium adsorption into the biofilm

The affinity present at the active site of the biofilm polymer is equal to the opposite ions to maintain electroneutrality; this site is offset by the charge absorbed from the environment, equal to the number of adsorption sites. The higher the adsorption site, the higher the adsorption charge [25, 26].

Adsorption effectiveness showed a significant decrease from 57.7% to 26.6% in line with an increase in the initial concentration of chromium ions from 10 to 1000 mg/L (Figure 3). This phenomenon suggests that the adsorption process is highly dependent on the ratio between the number of heavy metal ions in the solution and the number of active sites available on the surface of the biofilm. At low initial concentrations, the number of active sites is still sufficient to bind most of the chromium ions present, so the level of adsorption efficiency is relatively high. Conversely, when the concentration of chromium increases significantly, the availability of the active site becomes limited compared to the number of ions that must be adsorbed, leading to a decrease in the proportion of ions that successfully adhere to the surface of the biofilm [8, 27].

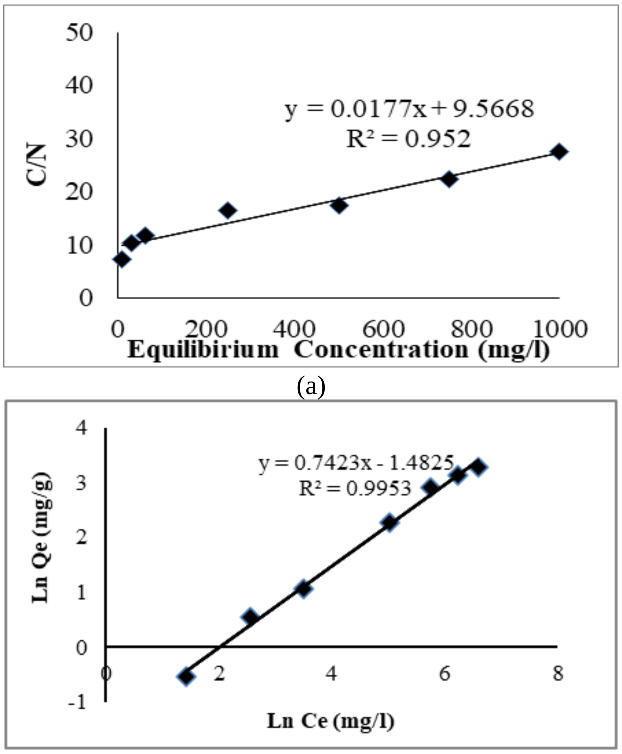
In the Langmuir isotherm model, adsorption is assumed to occur on a homogeneous surface with a limited number of active sites, where each site can only be occupied by one molecule thus forming a single layer [23], and there is no interaction between adsorbed molecules; As the concentration of the adsorbate increases, the number of molecules occupying the active site will increase until the entire site is filled and reaches a state of saturation [28], so that once it approaches saturation, the addition of concentration no longer significantly increases the amount of the adsorbed substance and causes a decrease in relative efficiency [15].

3.3 Adsorption model

Furthermore, to evaluate the adsorption mechanism of chromium (Cr) onto the biofilm, the linearized form of the Langmuir isotherm model was applied. The calculated C/N values increased with rising equilibrium Cr concentrations (C). Specifically, at 10 mg L^{-1}, the C/N value was 7.331 L g^{-1}; at 30 mg L^{-1}, it increased to 10.362 L g^{-1}; at 62.5 mg L^{-1}, 11.679 L g^{-1}; at 250 mg L^{-1}, 16.469 L g^{-1}; at 500 mg L^{-1}, 17.357 L g^{-1}; at 750 mg L^{-1}, 22.362 L g^{-1}; and at 1000 mg L^{-1}, it reached 27.540 L g^{-1}. The Langmuir isotherm plot is presented in Figure 4(a), while the Freundlich isotherm plot is presented in Figure 4(b).

These results indicate a progressive increase in the C_e/q_e ratio with increasing Cr concentration, reflecting changes in adsorption behavior. At lower concentrations, the biofilm surface provides abundant active binding sites, facilitating efficient interaction with Cr ions. However, as the concentration increases, these active sites gradually become saturated, leading to a reduced adsorption efficiency. This trend is consistent with the fundamental assumption of the Langmuir model, which describes monolayer adsorption onto a homogeneous surface with a finite number of binding sites.

The observed linear relationship between C/N and C enables the determination of key Langmuir parameters, including the maximum adsorption capacity (N_{max}) and the Langmuir affinity constant (b), which represent the adsorption capacity and affinity of the biofilm toward Cr ions, respectively [15].



(a)

(b)

Figure 4. Relationship of C/N to Cr metal balance in biofilm using (a) the Langmuir Model and (b) the Freundlich model

The value of $R^2 = 0.952$ indicates that the Langmuir isothermal model used has an excellent degree of compatibility with the Cr adsorption data by biofilm, since approximately 95.20% of the variation of the experimental data can be explained by the linear regression line of the Langmuir equation. In isothermal adsorption studies, the model is considered suitable if the R^2 value is close to 1 when compared to the Freundlich model, so that a value of 0.952 (Langmuir) indicates that the Cr adsorption process in biofilms tends to follow a monolayer mechanism on homogeneous surfaces according to Langmuir's assumption and is reliable for determining the parameters of maximum adsorption capacity and equilibrium constants [29].

From the linearized Langmuir plot, the maximum adsorption capacity (N_{max}) was determined to be 56.50 mg/g, and the Langmuir affinity constant (b) was 0.00185 L/mg. These parameters were derived from the slope ($1/N_{max}$) and y-intercept ($1/N_{max} \cdot b$) of the linear regression of C/N versus C (Figure 4(a)), confirming monolayer adsorption on a homogeneous biofilm surface.

4. CONCLUSION

The natural biofilm that grows on the rocks in the Manyar River, Gresik, has excellent potential as a biosorbent to reduce chromium metal (Cr) pollution in the waters. The adsorption process proceeds very quickly, as indicated by the optimum contact time of only 5 minutes and the adsorption capacity of 4.390 – 4.405 mg/g. Isothermal analysis showed that the adsorption mechanism followed the Langmuir Isothermal model with a determination coefficient value (R^2) of 0.952, b of 0.00185 L/mg (derived from the slope and intercept of the linearized Langmuir equation), and a maximum capacity (N_{max}) of 56.50 mg/g, which indicated adsorption in the form of a single layer (monolayer) on the surface of the biofilm. Thus, the natural biofilm from the Manyar River can be considered as an effective, economical, and environmentally friendly alternative in efforts to handle heavy metal pollution, especially chromium, in the aquatic environment.

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