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# Binary Biosorption of Cu(II) and Cr(VI) by Naturally Formed Biofilm Matrices

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Water pollution poses a significant ecological threat, contributing to the widespread degradation of aquatic ecosystems. Among the pollutants, heavy metals pose severe risks to both organisms and human health, emphasizing the urgent need for effective pollution control technologies. Biosorption presents a promising, cost-effective, and environmentally friendly solution to mitigate heavy metal contamination. Biofilms, consisting of microbial communities, have emerged as potential biosorbents for heavy metals. Since heavy metals can exist as cations and anions concurrently in aquatic environments, understanding their behavior in binary systems is essential for biosorption strategies. This study focuses on the binary biosorption of Cu(II) and Cr(VI), representing cationic and anionic heavy metals. Specific adsorption sites are identified by analyzing adsorption kinetics, isotherms, and IR spectra of biofilms. The results indicate that biofilms, in a binary scenario, demonstrate nearly identical adsorption capacities for Cu(II) and Cr(VI). Moreover, the adsorption process follows the Langmuir adsorption model, emphasizing the potential of biofilms as effective biosorbents for the simultaneous removal of cationic and anionic heavy metals. This study advocates for a sustainable approach to heavy metal remediation through the utilization of biofilms.

**Keywords:** adsorption, aquatic ecosystem, biofilm, binary system, microbial ecology.

## Introduction

Wastewater discharged by various industries significantly contributes to environmental pollution (Ahmad et al., 2008). Heavy metals, prevalent within aquatic environments, pose substantial risks to humans, plants, and animals (Quintelas et al., 2009; Yanuar

et al., 2021). These metals migrate and accumulate within the aquatic food chain, exacerbating their ecological impact (Febriana et al., 2011; Takahashi et al., 2012). Therefore, effective removal or immobilization of these contaminants is essential to safeguard aquatic

ecosystems (Wang and Chen, 2009; Mavakala et al., 2022).

Among the prevalent heavy metals, copper and chromium play pivotal roles in various industries. Copper is widely used in cables, plumbing, corrosion-resistant coatings, and automotive components (Bilgic et al., 2022). Conversely, chromium is found to be extensively used in metal plating, leather tanning, and electroplating processes (Ulfa et al., 2019). Elevated levels of Cu(II) and Cr(VI) can result in severe health issues, sometimes proving fatal (Jiang et al., 2020; Ju et al., 2022). In natural aquatic environments, copper primarily exists as Cu(II) cations, while chromium exists as  $\text{Cr}_2\text{O}_7^{2-}$  or Cr(VI) anions at pH above 6.5. The coexistence of cationic and anionic heavy metals like Cu(II) and Cr(VI) underscores the urgent need for technology capable of concurrently mitigating pollution from both ion types.

Biosorption is a promising technology for purifying pollutants, particularly heavy metals (Kurniawan et al., 2020). Its potential lies in its ability to provide a cost-effective and environmentally friendly solution that is easily applicable. The effectiveness of biosorption hinges significantly on the choice of biosorbent, with aquatic microbes, particularly those inhabiting biofilms, garnering attention as potential candidates (Zhao et al., 2022). While some studies have explored the utility of biofilms as biosorbents (Jasu and Ray, 2021; Mustafa et al., 2021), limited research has delved into binary biosorption employing naturally formed biofilms. Given the complexity of water contamination involving multiple heavy metals, the simultaneous biosorption of cationic and anionic heavy metals, such as Cr(VI) and Cu(II), within a binary system using biofilms is of utmost significance. This study aims to address this critical knowledge gap.

This study delves into the intricate dynamics of binary biosorption using naturally formed biofilms when challenged with Cu(II) and Cr(VI). The findings reveal that biofilms exhibit remarkable equitability in adsorbing Cu(II) and Cr(VI) within this binary biosorption framework. Moreover, this adsorption occurs rapidly and closely adheres to the Langmuir adsorption model. These outcomes unequivocally underscore the potential of biofilms as highly promising biosorbents for the concurrent removal of cationic and anionic heavy metals in binary biosorption scenarios.

## Methods

### Biofilm sample preparation

The biofilms utilised in this study were sourced from natural biofilms thriving on stones within the freshwater pool at the University of Brawijaya in Malang City, Indonesia. Stones adorned with biofilm matrices were carefully transferred into plastic containers and filled with pond water. These containers, housing biofilm and the surrounding water, were transported to the laboratory while maintaining a temperature below 4°C. The biofilm was harvested from the stone surfaces using a sterile toothbrush and then suspended in 40 mL of distilled water. Subsequently, biofilm samples were processed into pellets via centrifugation at 8000 x g for 3 minutes. These biofilm samples were stored at -40°C until required for the experiment.

### Kinetics of adsorption

Copper [Cu(II)] and Chromium [Cr(VI)] solutions were prepared by dissolving reagent-grade  $\text{CuCl}_2$  and  $\text{K}_2\text{Cr}_2\text{O}_7$  in distilled water. Subsequently, 0.5 grams of biofilm pellets were introduced into a glass beaker containing 50 mL of a solution containing 50 mg/L of  $\text{CuCl}_2$  and  $\text{K}_2\text{Cr}_2\text{O}_7$ . The resulting suspension was thoroughly homogenized using a magnetic stirrer. At specific time intervals (5, 15, 30, 60, 120, and 180 minutes), 4 mL of the solution was extracted and then centrifuged at 8000 x g for 3 minutes to separate the pellet from the supernatant. Concentration measurements for Cr(VI) and Cu(II) in the supernatant were carried out using a UV-VIS Spectrophotometer. The adsorption process was assessed by calculating ion concentration differences between the supernatant and the control (i.e., a solution lacking biofilm). Notably, this experiment was independently repeated three times.

### Adsorption isotherm

Several solutions of Cu(II) and Cr(VI) with different concentrations were prepared by dissolving reagent grades of  $\text{CuCl}_2$  and  $\text{K}_2\text{Cr}_2\text{O}_7$  simultaneously in distilled water. The concentrations were 125, 250, 1000, and 2000 mg/L. Then, 0.5 grams of biofilm pellet was added to Erlenmeyer containing 50 mL of Cu(II) and Cr(VI) solution with different concentrations (i.e., 125, 250, 1000, and 2000 mg/L) and mixed well using a magnetic stirrer. After 10 minutes, the biofilm suspensions were centrifuged (10 000 x g for 3 minutes) to separate the biofilm and supernatant. The Cu(II) and Cr(VI) concentrations in the supernatant were measured using

a UV–VIS Spectrophotometer. The number of ions adsorbed was calculated from the difference between the concentrations in the supernatant and the control (solution without biofilm). The experiment was repeated three times, independently.

Adsorption isotherm of Cu(II) and Cr(VI) into the biofilm matrices was analysed using the Langmuir model shown in equation 1.

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

Equation 1, presented above, posits the existence of a dynamic equilibrium between the ions within the adjacent water of the biofilm matrices and the adsorbed ions within the biofilm matrices (N). This equilibrium condition ultimately leads to attaining the equilibrium concentration (C). The adsorption and desorption rates ratio is elucidated by the adsorption equilibrium constant (b), typically measured in L/mg. When the adsorption rate surpasses the desorption rate, the value of b increases. A plot of C/N against C yields a linear graph characterized by a slope of 1/N<sub>max</sub> and a y-intercept of 1/(N<sub>max</sub>)b. Consequently, this allows for determining the values of N<sub>max</sub> and b.

### FTIR analysis

Copper [Cu(II)] and chromium [Cr(VI)] solutions were prepared by dissolving reagent-grade CuCl<sub>2</sub> and K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> in distilled water. Subsequently, 0.5 grams of biofilm pellets were introduced into a glass beaker containing 50 mL of a solution containing 50 mg/L of CuCl<sub>2</sub> and K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>. The resulting suspension was thoroughly homogenized using a magnetic stirrer. At specific time intervals (5, 15, 30, 60, 120, and 180 minutes), 4 mL of the solution was extracted and then centrifuged at 8000 x g for 3 minutes to separate the pellet from the supernatant. Concentration measurements for Cr(VI) and Cu(II) in the supernatant were carried out using a UV–VIS Spectrophotometer. The adsorption process was assessed by calculating ion concentration differences between the supernatant and the control (i.e., a solution lacking biofilm).

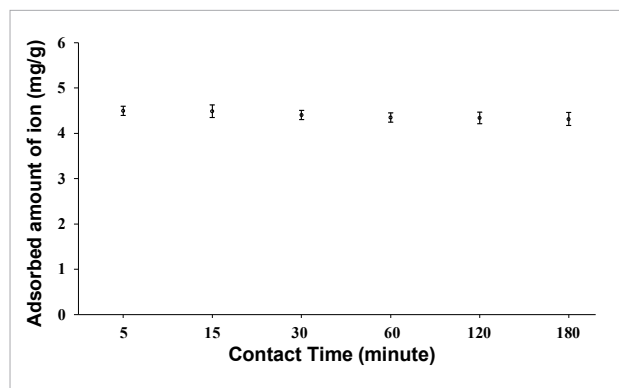
## Results and Discussion

### Kinetics of adsorption

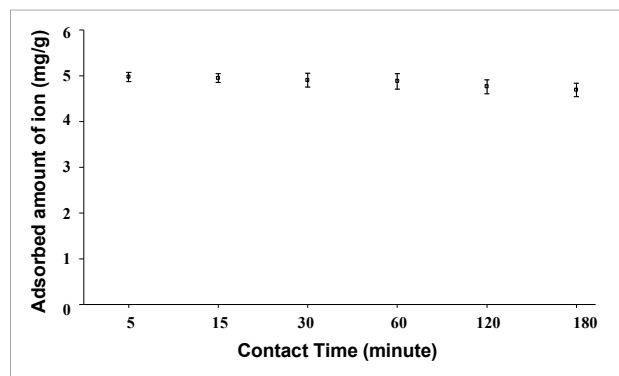
The kinetics of Cu(II) and Cr(VI) adsorption within binary systems by biofilms, spanning contact times from 5 to

180 minutes, are depicted in Figs. 1 and 2, respectively. Notably, Cr(VI) adsorption remained consistent from the initial 5 minute mark throughout the experiment, plateauing at approximately 4.9 mg/g. This trend was mirrored in the case of Cu(II) adsorption, where the uptake reached a stable level of approximately 4.4 mg/g and remained consistent over 180 minutes. This rapid and sustained adsorption process reflects a distinctive feature of the passive accumulation of ions via physicochemical mechanisms.

**Fig. 1.** Time course of Cu(II) adsorption into biofilm. Bars represent the standard error



**Fig. 2.** Time course of Cr(VI) adsorption into the biofilm. Bars represent the standard error



The physicochemical process governing ion adsorption into biofilm matrices is a fundamental aspect of passive uptake, operating independently of microbial metabolic activity (Choi and Kan, 2019; Nandi et al., 2021). Under this mechanism, adsorption occurs due to the attractive electrostatic interactions between ions and the charged sites on biofilm polymers (Ulfa et al., 2019). These charged sites serve as binding sites, facilitating the adherence of ions to the biofilm matrix.

The significance of this physicochemical mechanism becomes particularly evident when biofilms are exposed to the simultaneous adsorption of various heavy metal types, encompassing both cationic and anionic variants. The consistent adsorption behavior observed for both Cu(II) and Cr(VI) in our study, regardless of their distinct charge characteristics, supports the effectiveness of this process in accommodating a broad spectrum of heavy metal ions. This versatility underscores the potential utility of biofilms as robust and versatile biosorbents for removing complex mixtures of heavy metals in aquatic environments.

The attractive electrostatic interactions between ions and biofilm polymers play a pivotal role in facilitating the binding of heavy metals to the biofilm matrix. This electrostatic interaction process is well-established in the literature, and its dominance in our study reaffirms its crucial role in the adsorption of heavy metals by biofilms. Overall, the study provides valuable insights into the physicochemical mechanisms underpinning the biosorption of heavy metals, particularly in a binary system, reinforcing the versatility and potential of biofilms as effective biosorbents.

The kinetics of Cu(II) and Cr(VI) adsorption within the binary systems exhibit comparable characteristics. Both ions undergo swift adsorption, with the amount adsorbed remaining relatively stable. Consequently, it is plausible to deduce that both ions are assimilated into the biofilm matrices through passive uptake mechanisms. This mechanism is likely underpinned by electrostatic interactions and ion exchange processes (Kurniawan and Yamamoto, 2022).

To compare the adsorption characteristics of Cu(II) and Cr(VI), the kinetics of adsorption data for both ions were

subjected to independent t-tests. The results, presented in *Table 1*, revealed a  $P$  value  $< 0.001$ , indicating a statistically significant difference between the adsorption processes of Cu(II) and Cr(VI) into biofilm matrices. Although the average difference in adsorption amounts between Cu(II) and Cr(VI) is relatively small (4.4 mg/g and 4.9 mg/g, respectively), suggesting a similar trend, distinctions in their adsorption characteristics by biofilms persist.

These differences likely arise from variations in the electrostatic properties between cationic and anionic heavy metals. One notable distinction is the deposition of these ions within different regions of the biofilm matrices. Cationic heavy metals tend to accumulate in areas rich in negatively charged sites, whereas anionic heavy metals preferentially bind to regions abundant in positively charged sites.

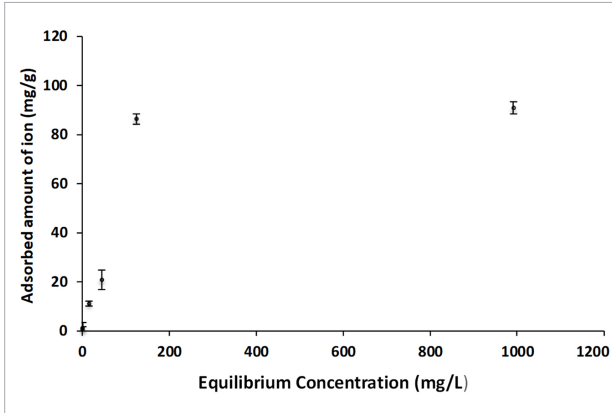
### Adsorption isotherm

To explore Cu(II) and Cr(VI) biosorption behavior within the binary system, we analyzed their adsorption isotherms in *Figs. 3* and *4*. Utilizing insights from the kinetic adsorption experiment, we maintained a contact time of 10 minutes for this analysis. Interestingly, we observed a gradual increase in Cu(II) and Cr(VI) adsorption with rising concentrations, reaching saturation at higher levels. The adsorption data obtained in this study exhibit the characteristic L type of adsorption isotherm, indicating a specific adsorption isotherm model. This behavior likely arises from the variable availability of active biofilm sites for Cu(II) and Cr(VI) adsorption within the binary system. The higher abundance of active sites at lower concentrations facilitates enhanced ion accumulation.

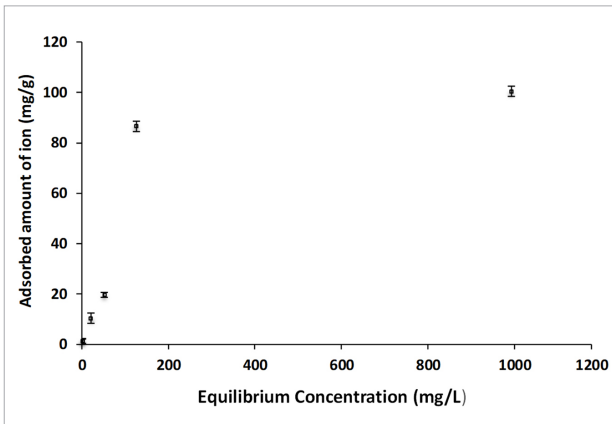
**Table 1.** Independent t-test results to compare the kinetics of adsorption of Cu(II) and Cr(VI) into biofilms

		Levene's test for equality of variances		t-test for equality of means							
		F	ig.	t	df	Significance		Mean difference	Std. error difference	95% confidence interval of the difference	
						One sided P	Two sided P			Lower	Upper
VAR00005	Equal variances assumed	2.990	093	-15.089	34	< 0.001	< 0.001	-0.46051	0.03052	-0.52253	-0.39848
	Equal variances not assumed			-15.089	30.629	< 0.001	< 0.001	-0.46051	0.03052	-0.52278	-0.39823

**Fig. 3.** Adsorption isotherm of Cu(II) into biofilm. Bars represent the standard error



**Fig. 4.** Adsorption isotherm of Cr(VI) into biofilm. Bars represent the standard error



A comparison of the adsorption isotherms of Cu(II) and Cr(VI) by the biofilm matrices was performed using a two-sample independent t-test. Here,  $\mu_1$  represents the population mean of the equilibrium concentration of Cu(II), while  $\mu_2$  represents the population mean of the equilibrium concentration of Cr(VI). The results of the test are shown in Table 2. The obtained *P* value from this test is 0.992, which exceeds 0.05. This indicates no significant difference in the equilibrium concentration between Cu(II) and Cr(VI) adsorption by the biofilm matrices in the binary system. Both exhibit a trend consistent with the L type of adsorption, where the adsorption capacity increases with the rise in equilibrium concentration and eventually stabilizes at higher concentrations.

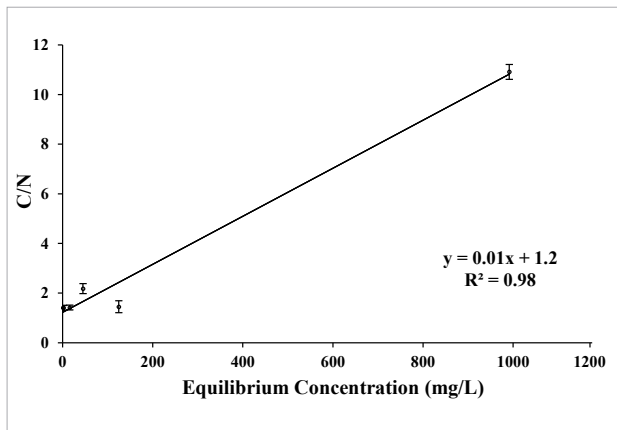
In order to deepen our comprehension of the biosorption traits of Cu(II) and Cr(VI), we employed the

**Table 2.** Results of the two-sample independent t-test comparing the adsorption isotherms of Cu(II) and Cr(VI) into biofilms

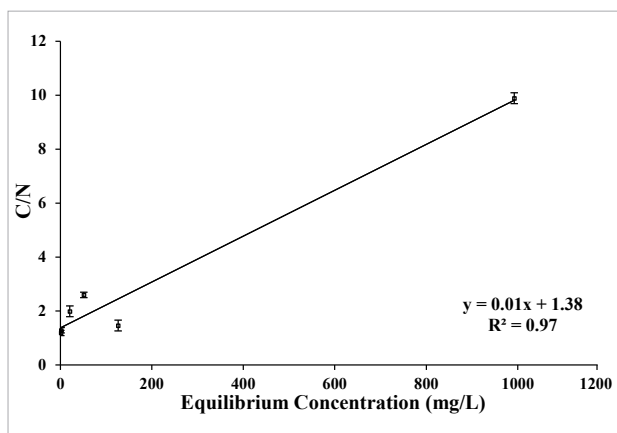
Null hypothesis		$H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis		$H_1: \mu_1 - \mu_2 \neq 0$
T-Value	DF	P Value
-0.01	10	0.992

Langmuir isotherm model (refer to Figs. 5 and 6). Within this model, we graphed the ratio of the amount of Cr(VI) and Cu(II) adsorbed into the biofilm (*N*; mg/g) against the equilibrium concentration (*C*; mg/L). This approach facilitated estimating the maximum biosorption capacity (*N*<sub>max</sub>; mg/g) and the adsorption equilibrium constant (*b*; L/mg).

**Fig. 5.** Plotting Cu(II) adsorption results in the Langmuir isotherm model. Bars represent the standard error



**Fig. 6.** Plotting Cr(VI) adsorption results in the Langmuir isotherm model. Bars represent the standard error



The adsorption of Cu(II) and Cr(VI) into the biofilms conforms well to the Langmuir isotherm model ( $R^2 = 0.98$  and  $0.97$  for Cu(II) and Cr(VI), respectively), indicating monolayer adsorption predominance. This process primarily involves the interaction between these ions and charged sites within the biofilm, facilitated by attractive electrostatic interactions with biofilm polymers (Kurniawan et al., 2012; Gadd, 2009; Chubar et al., 2008; Buhani et al., 2006).

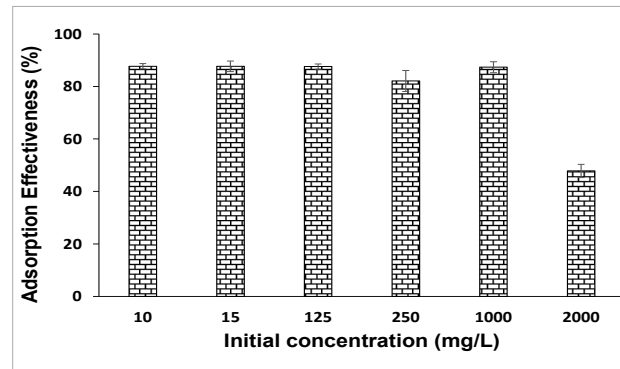
The maximum uptake of Cu(II) and Cr(VI) by the biofilm ( $N_{max}$ ) was estimated at 102.04 mg/g and 100 mg/g, respectively, implying a slightly greater affinity for anionic variants. This capability arises from the presence of positively and negatively charged sites in biofilm matrices (Kurniawan and Fukuda, 2022), facilitating simultaneous adsorption of Cu(II) and Cr(VI). The marginally higher adsorption of anionic heavy metals may stem from more adsorption sites or spaces between biofilm polymers, enhancing accessibility.

The equilibrium constants ( $b$ ) for Cu(II) and Cr(VI) were determined to be 0.008 L/mg and 0.007 L/mg, respectively, suggesting nearly equal binding strength within the biofilm matrix. Cu(II) exhibits slightly stronger binding, likely due to biofilm matrices' net negative charge around pH 7, rendering cationic bonds marginally more robust (Kurniawan and Fukuda, 2022). This study underscores the biofilm matrix's capability to adsorb cationic and anionic heavy metals simultaneously within a binary biosorption system, emphasizing comparable adsorption capacity and strength for both ion types.

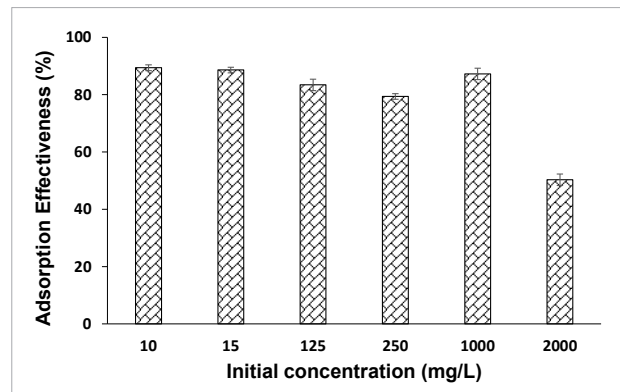
Furthermore, the adsorption efficiency of Cu(II) and Cr(VI) by the biofilm matrices was evaluated based on initial concentration data in the adsorption isotherm experiment, revealing a notable pattern. Efficiency gradually diminishes with increasing Cu(II) and Cr(VI) concentrations. For instance, Cr(VI) efficiency peaks at 87.3% within the 125–1000 mg/L concentration range, dropping to 50.3% at 2000 mg/L. Similar trends are observed with Cu(II), with efficiency peaking at 87.6% within the concentration range of 125–1000 mg/L but declining to 47.8% at 2000 mg/L.

These findings highlight the intricate relationship between concentration and biosorption efficiency. Elevated concentrations enhance ion adsorption by increasing active site availability, but eventually, a saturation point is reached, leading to diminished efficiency. This decline may arise from limited active sites, competition

**Fig. 7.** Effectiveness of Cu(II) adsorption by biofilm. Bars represent the standard error



**Fig. 8.** Effectiveness of Cr(VI) adsorption by biofilm. Bars represent the standard error



for adsorption sites, and potential ion interactions at higher concentrations. Understanding these dynamics is vital for optimizing biosorption processes and assessing their practical applicability in mitigating heavy metal contaminants in aquatic ecosystems.

The observed reduction in adsorption efficiency at higher concentrations likely results from decreased available active sites within the biofilm for Cu(II) and Cr(VI) adsorption in the binary system. This study unequivocally demonstrates the biofilm's remarkable efficacy in adsorbing heavy metals, even within a binary system. Therefore, establishing efficiency thresholds is essential when considering the application of biofilms as biosorption agents for Cu(II) and Cr(VI) in binary systems for water treatment.

A comparative analysis of the adsorption efficiency of Cu(II) and Cr(VI) by the biofilm was conducted using a two-sample independent t-test (Table 3). Here,  $\mu_1$  represents the population mean of Cu(II) adsorption



**Table 3.** Results of the two-sample independent *t*-test comparing the adsorption efficiency of Cu(II) and Cr(VI) into biofilms

Null hypothesis		$H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis		$H_1: \mu_1 - \mu_2 \neq 0$
<b>T value</b>	<b>DF</b>	<b>P value</b>
-0.01	10	0.873

efficiency, while  $\mu_2$  represents the population mean of Cr(VI) adsorption efficiency. The analysis resulted in a *t*-value of 0.16 and a *P* value of 0.837. Given that the *P* value exceeds 0.05, it suggests no significant difference in the adsorption efficiency between Cu(II) and Cr(VI) by the biofilm. This finding indicates that the biofilm matrices exhibit nearly identical efficiency levels in the binary system adsorbing Cu(II) and Cr(VI). Consequently, biofilm matrices demonstrate the capability to adsorb cationic and anionic heavy metals concurrently and with comparable efficiency. This adsorption characteristic underscores the potential effectiveness of biofilm matrices as biosorbents for treating wastewater containing cationic and anionic heavy metals.

Furthermore, we conducted a detailed analysis of Cu(II) and Cr(VI) adsorption efficiency by biofilm matrices in binary systems using cluster analysis with the Euclidean distance method and complete linkage (Fig. 9). The

outcomes revealed a distinct pattern in the adsorption efficiency of Cu(II) and Cr(VI), categorized into two clusters. The first cluster encompassed adsorption at initial concentrations of 10, 15, 125, 250, and 1000 mg/L, while the second cluster represented adsorption at an initial concentration of 2000 mg/L.

These results highlight the uniformity in adsorption efficiency for Cu(II) and Cr(VI) at concentrations of 10, 15, 125, 250, and 1000 mg/L. However, a significant deviation in efficiency was observed at a concentration of 2000 mg/L. This suggests that while the adsorption efficiency by biofilm matrices remains consistently high at low to moderate concentrations, it diminishes at higher concentrations, such as 2000 mg/L.

These insights are invaluable for developing environmental purification technologies based on the adsorption process of cationic and anionic heavy metals by biofilm matrices. By understanding the concentration-dependent nature of adsorption efficiency, we can tailor purification methods to achieve optimal performance and desired efficiency levels.

### FTIR spectra

FTIR analysis was utilized to identify the active functional groups involved in the biosorption process of Cr(VI) and Cu(II) within binary systems by biofilms (Fig. 10).

**Fig. 9.** Dendrogram from cluster analysis with Euclidean distance method and complete linkage for Cu(II) and Cr(VI) adsorption by biofilm matrices

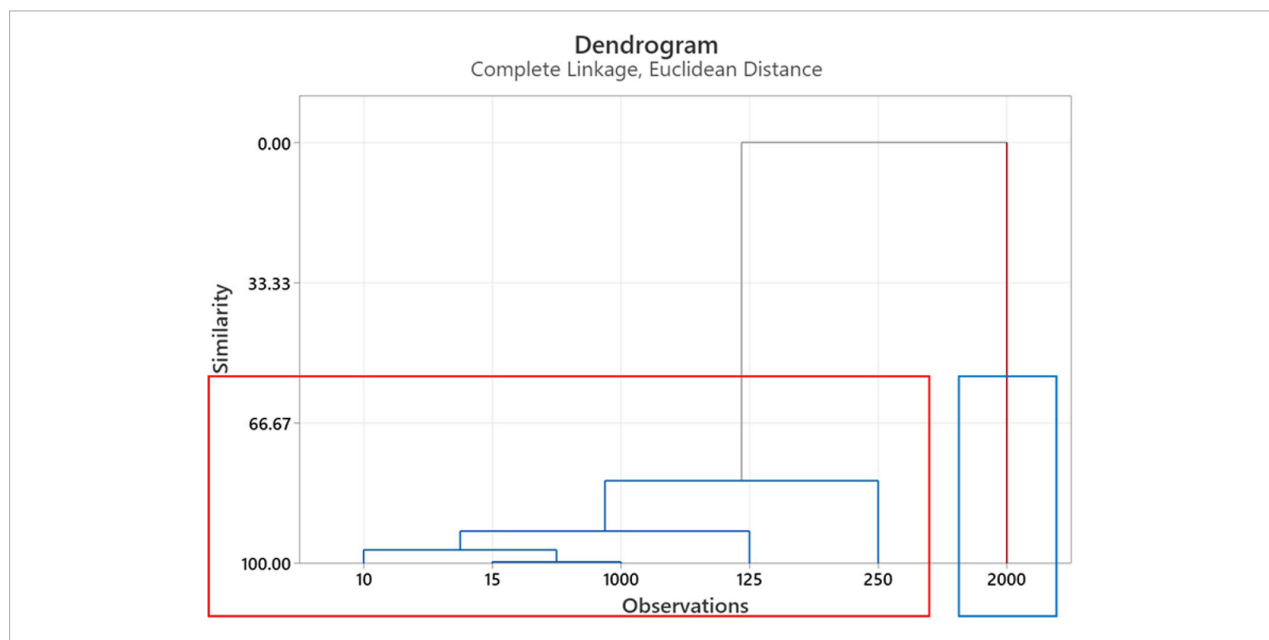


Fig. 10. FTIR spectra analysis of biofilms before and after adsorption of Cu(II) and Cr(VI) in a binary biosorption system



The analysis of biofilms using FTIR revealed distinct alterations in the biofilm composition before and after the adsorption of Cu(II) and Cr(VI). For instance, the wave peak observed at  $3402.562\text{ cm}^{-1}$  in the intact biofilm shifted to  $3430.730\text{ cm}^{-1}$  post-adsorption, indicating changes in stretching vibrations associated with O–H or N–H groups. The wave peak at  $2922.675\text{ cm}^{-1}$  also shifted to  $2926.279\text{ cm}^{-1}$ , indicating activity in the aliphatic C–H functional group. The presence of C=O-diketone functional groups was indicated by a change from a peak at  $1541.072\text{ cm}^{-1}$  to  $1540.986\text{ cm}^{-1}$ . Furthermore, the transition from  $1456.309\text{ cm}^{-1}$  to  $1384.661\text{ cm}^{-1}$  in the wave peak suggested the presence of aromatic nitro functional groups. In comparison, the shift from  $1268.310\text{ cm}^{-1}$  to  $1113.342\text{ cm}^{-1}$  indicated the presence of the alkyl halide functional group, possibly caused by C–X stretching. The transition from  $1057.025\text{ cm}^{-1}$  to  $949.19\text{ cm}^{-1}$  signified the involvement of OH, CH, C–OH, and CH<sub>2</sub> on the glycosyl unit in the adsorption process.

The stretching of O–H alcohol typically occurs within the  $3200\text{--}3600\text{ cm}^{-1}$  region, while alpha CH vibrations are observed within the  $3000\text{--}2700\text{ cm}^{-1}$  range, characteristic of alkane and alkyl groups. The stretching form of C=C alkenes becomes evident in the  $1645\text{--}1670\text{ cm}^{-1}$  range, with CO displaying activity within the  $1050\text{--}1200\text{ cm}^{-1}$  region (Anam et al., 2007). The alterations observed in the IR-spectra profiles of the biofilm matrices before and after the adsorption of Cu(II) and Cr(VI) suggest the involvement of functional groups

such as amino and carboxyl groups in the biofilm polymers' binding of heavy metals. This study indicates that the adsorption of cationic and anionic heavy metals is facilitated by the presence of ionized functional groups within the biofilm, offering positively and negatively charged sites for ion binding.

These findings offer critical insights into the mechanisms underpinning the adsorption of Cu(II) and Cr(VI) by biofilms, confirming the role of specific functional groups in facilitating this process. Biofilms' ability to accommodate diverse heavy metals, encompassing cationic and anionic varieties, underscores their potential as versatile and practical biosorbents for removing heavy metals from aqueous environments.

## Conclusions

In this study, we investigated the dynamics of binary biosorption, explicitly examining the interactions between Cu(II) and Cr(VI) within biofilm matrices – representing cationic and anionic heavy metals, respectively. Our results reveal the remarkable capacity of biofilm matrices to adsorb these ions concurrently within a binary system. This intricate process is governed by complex physicochemical mechanisms involving interactions between ions and the charged sites distributed across the biofilm matrices. Cu(II) and Cr(VI) demonstrated similar adsorption behavior within the binary systems. The nearly equivalent adsorption of these



ions, with a slight preference for Cr(VI), highlights the adaptable nature of biofilms in accommodating heavy metals with diverse charge properties. Moreover, the comparable binding strengths observed for cationic and anionic ions within the biofilm matrix, albeit with a slight preference for cationic metals, suggest the involvement of ionized functional groups within the biofilm structure. These groups serve as charged binding sites, facilitating the adsorption of both types of heavy metals. Our findings carry significant implications for environmental remediation endeavors, emphasizing the considerable potential of biofilm matrices as effective biosorbents for addressing the complex challenges posed by heavy metal pollution in aquatic ecosystems.

## References

- Ahmady-Asbchin S., Andrès Y., Gérente C., Le Cloirec P. (2008) Biosorption of Cu (II) from aqueous solution by *Fucus serratus*: surface characterization and sorption mechanisms. *Biore-sour Technol* 99(14): 6150-6155. <https://doi.org/10.1016/j.biortech.2007.12.040>
- Bilgic A., Cimen A., Kursunlu A.N. (2022) "Killing two birds with one stone": A fluorescent hybrid nanoparticle modified with BODIPY for efficiently detection and removal of toxic Cu (II) ion from aqueous solutions. *Sci Total Environ* 845: 157170. <https://doi.org/10.1016/j.scitotenv.2022.157170>
- Buhani B., Suharso S., Sembiring Z. (2006) Biosorption of Metal Ions Pb(II), Cu(II), and Cd(II) on *Sargassum Duplicatum* Immobilized Silica Gel Matrix. *Indones J Chem* 6(3):245-250. <https://doi.org/10.22146/ijc.21726>
- Choi Y.K., Kan E. (2019) Effects of pyrolysis temperature on the physicochemical properties of alfalfa-derived biochar for the adsorption of bisphenol A and sulfamethoxazole in water. *Chemosphere* 218: 741-748. <https://doi.org/10.1016/j.chemosphere.2018.11.151>
- Chubar N., Behrends T., Van Cappellen P. (2008) Biosorption of metals (Cu<sup>2+</sup>, Zn<sup>2+</sup>) and anions (F<sup>-</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>) by viable and autoclaved cells of the Gram-negative bacterium *Shewanella putrefaciens*. *Colloids Surf B Biointerfaces* 65(1): 126-133. <https://doi.org/10.1016/j.colsurfb.2008.03.006>
- Febriana S.A., Jungbauer F., Soebono H., Coenraads P.J. (2012) Inventory of the chemicals and the exposure of the workers' skin to these at two leather factories in Indonesia. *Int Arch Occup Environ Health* 85(5): 517-526. <https://doi.org/10.1007/s00420-011-0700-1>
- Gadd GM (2009). Biosorption: a critical review of scientific rationale, environmental importance and significance for pollution treatment. *J Chem Technol Biotechnol: International Research in Process, Environmental and Clean Technology* 84(1): 13-28. <https://doi.org/10.1002/jctb.1999>
- Jasu A., Ray R.R. (2021) Biofilm mediated strategies to mitigate heavy metal pollution: A critical review in metal bioremediation. *Biocatal Agric Biotechnol* 37: 102183. <https://doi.org/10.1016/j.bcab.2021.102183>
- Jiang Y., Liu Y., Zhang H., Yang K., Li J., Shao S. (2020) Aerobic granular sludge shows enhanced resistances to the long-term toxicity of Cu(II). *Chemosphere* 253: 126664. <https://doi.org/10.1016/j.chemosphere.2020.126664>
- Ju L., Jiao Z., Ge S., Zhan W., Liu Y., Ren Q., Liao Q., Yang Z., Wang Y. (2022) Formation, stability and mobility of soluble Cr(III) during Cr(VI) reduction by *Pannonibacter phragmitetus* BB. *Environ Technol Innov* 27: 102496. <https://doi.org/10.1016/j.eti.2022.102496>
- Kurniawan A., Fukuda Y. (2022) Analysis of the electric charge properties of biofilm for the development of biofilm matrices as biosorbents for water pollutant. *Energy Ecol Environ* 2022. <https://doi.org/10.1007/s40974-022-00253-6>
- Kurniawan A., Salamah L.N., Amin A.A., Yanuar A. (2020) Biosorption of Cu(II) by natural biofilm matrix of Lahor Reservoirs, Indonesia. In *IOP Conference Series: Earth and Environmental Science* 493(1): 012008. <https://doi.org/10.1088/1755-1315/493/1/012008>
- Mavakala B.K., Sivalingam P., Laffite A., Mulaji C.K., Giuliani G., Mpiana P.T., Poté J. (2022). Evaluation of heavy metal content and potential ecological risks in soil samples from wild solid waste dumpsites in developing country under tropical conditions. *Environ Chall* 7: 100461. <https://doi.org/10.1016/j.envc.2022.100461>
- Mustafa S., Bhatti H.N., Maqbool M., Iqbal M. (2021) Microalgae biosorption, bioaccumulation and biodegradation efficiency for the remediation of wastewater and carbon dioxide mitigation:

- Prospects, challenges and opportunities. *J Water Process Eng* 41: 102009. <https://doi.org/10.1016/j.jwpe.2021.102009>
- Nandi D., Ghosh S.K., Ghosh A., Siengchin S., Roy A., Gupta K., Parameswaranpillai J., Bhowmick A.K., Ghosh U.C. (2021) Arsenic removal from water by graphene nanoplatelets prepared from nail waste: A physicochemical study of adsorption based on process optimization, kinetics, isotherm and thermodynamics. *Environ Nanotechnol Monit Manag* 16: 100564. <https://doi.org/10.1016/j.enmm.2021.100564>
- Quintelas C., Rocha Z., Silva B., Fonseca B., Figueiredo H., Tavares T. (2009) Removal of Cd(II), Cr(VI), Fe(III) and Ni(II) from aqueous solutions by an *E. coli* biofilm supported on kaolin. *Chem Eng J* 149(1-3): 319-324. <https://doi.org/10.1016/j.cej.2008.11.025>
- Takahashi C.K., Turner A., Millward G.E., Glegg G.A. (2012) Persistence and metallic composition of paint particles in sediments from a tidal inlet. *Mar Pollut Bull* 64(1): 133-137. <https://doi.org/10.1016/j.marpolbul.2011.10.010>
- Ulfa S.M., Chamidah N., Kurniawan A. (2019) Adsorption of Cu (II) in aqueous solution by modified sawdust cellulose. *IOP Conference Series: Earth and Environmental Science* 239(1): 012008. <https://doi.org/10.1088/1755-1315/239/1/012008>
- Wang J., Chen C. (2009) Biosorbents for heavy metals removal and their future. *Biotechnol Adv* 27(2): 195-226. <https://doi.org/10.1016/j.biotechadv.2008.11.002>
- Yanuar A.T., Ilmi N., Amin A.A., Pramudia Z., Susanti Y.A.D., Salamah L.N., Kurniawan A. (2021) Analysis of biofilms as a biomonitoring agent for Cr (VI) pollution in Cokro River, Malang Regency. *AIP Conference Proceedings* 2353(1): 030102. <https://doi.org/10.1063/5.0053066>
- Zhao J., Ni G., Piculell M., Li J., Hu Z., Wang Z., Guo J., Yuan Z., Zheng M., Hu S. (2022) Characterizing and comparing microbial community and biofilm structure in three nitrifying moving bed biofilm reactors. *J Environ Manag* 320: 115883. <https://doi.org/10.1016/j.jenvman.2022.115883>

