Journal of Pharmaceuticals and Natural Sciences

B-CRETA Publisher
Pharma Insight Solutions

Article

Optimizing Sand–Based Biofilm Biosorption for Copper (Cu) Mitigation: Mechanisms, Optimal Conditions, and Environmental Implications

Rosna, **Ahmad Zaeni**, **Laode Kadidae**, **Zainal Syam Arifin***

Department of Chemistry, Faculty of Mathematic and Natural Science, Halu Oleo University, Kendari City, 93292 Southeast Sulawesi, Indonesia

* Correspondence: z.s.arifin@uho.ac.id

Citation: Rosna; Zaeni, A.; Kadidae, L.; Arifin, Z.S. Optimizing sand-based biofilm biosorption for copper (Cu) mitigation: Mechanism, optimal conditions, and environmental implications. J Pham Nat Sci 2025, 2(1), 37-46. https://doi.org/10.70392/jpns.v2i1.3746

Academic Editor: Dr. Herman

Received: 17 February 2025
Revised: 10 March 2025
Accepted: 17 March

Publisher's Note: B-CRETA publisher stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

ISSN: 3047-5457

Abstract

This study explores the use of sand-based biofilms for the biosorption of Cu ions, leveraging the natural ability of biofilms to adsorb and immobilize heavy metals. The research focuses on optimizing biosorption conditions, including contact time, initial Cu concentration, and pH, while evaluating the broader impacts on water quality parameters such as Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), and Total Dissolved Solids (TDS). The experimental results demonstrated that biosorption efficiency peaked at a contact time of 1 day, an initial Cu concentration of 100 mg/L, and a pH of 8. At these optimal conditions, the biofilm achieved a biosorption capacity of 39.7 mg/L for Cu ions. Moreover, the treatment significantly improved water quality, reducing COD by 77.06%, BOD by 78.92%, and TDS by 30%. The mechanism of biosorption was influenced by the availability of functional groups within the extracellular polymeric substance (EPS) of the biofilm, which provided binding sites for Cu ions. The influence of pH was particularly notable, as it regulated the ionic interactions between Cu^{2+} and the biofilm matrix. This study not only confirms the effectiveness of biofilm-based biosorption for heavy metal mitigation but also highlights its dual role in reducing organic and inorganic pollutants in wastewater. The use of sand as a substrate for biofilm growth adds an element of scalability and economic feasibility, making it an attractive solution for industrial applications. The findings underscore the potential of this eco-friendly approach to contribute to sustainable wastewater management, addressing both environmental and public health concerns associated with heavy metal contamination. Future research could explore the application of this method for other heavy metals and its integration into existing wastewater treatment systems.

Keywords: Biofilm; Biosorption; Copper (Cu); Heavy Metal; Wastewater

1. INTRODUCTION

Heavy metal pollution has become a significant global concern due to its persistence, bioaccumulation potential, and toxic effects on ecosystems [1–5] and human health [6–8]. Among various pollutants, copper (Cu) is a commonly found heavy metal in industrial effluents, including those from mining, metal plating, and electronics manufacturing [9–13]. Although Cu is essential for biological systems at trace levels, elevated concentrations can lead to severe ecological and health issues such as oxidative stress, neurotoxicity, and organ damage in humans [14–17].

Conventional methods for heavy metal removal, such as chemical precipitation, ion exchange, and activated carbon adsorption [18], have limitations, including high operational costs, inefficiency at low pollutant concentrations, and secondary pollution from chemical residues [19,20]. Biosorption, a process leveraging biological materials like microbial biofilms, has emerged as a cost-effective [21] and environmentally friendly alternative for heavy metal remediation [22,23]. Biofilms, microbial communities embedded within an extracellular polymeric substance (EPS), offer unique advantages for biosorption [24–29]. The EPS contains functional groups such as carboxyl (–COOH), hydroxyl (–OH), and amino (–NH_2) that play a crucial role in metal ion binding [30–33]. These groups interact with Cu^{2+} ions through electrostatic attraction, complexation, and ion exchange, enhancing biosorption efficiency [32,34,35]. Sand was chosen as a medium for biofilm cultivation due to its wide availability, low cost, and high surface area. This study aims to explore the optimal conditions for Cu biosorption using sand-based biofilms, including contact time, initial metal concentration, and pH. Furthermore, the study evaluates the broader environmental implications by analyzing changes in water quality parameters, namely COD, BOD, and TDS.

2. MATERIALS AND METHODS

2.1. Biofilm Cultivation

The biofilm was cultivated using sand as a medium in a bioreactor tank designed to optimize microbial growth. The bioreactor tank was made of glass with dimensions of 100 × 10 × 10 cm and did not have an aerator, relying solely on direct oxygen diffusion from the atmosphere. Due to the wastewater level being less than 10 cm, oxygen transfer occurred naturally at the air-water interface. Additionally, mixing within the bioreactor created turbulence, enhancing oxygen availability, though not as effectively as an aeration system. To simulate continuous flow conditions while operating in batch mode, an internal recirculation system (mimicking continuous flow) [36] was implemented. The bioreactor tank consisted of three main sections: a recirculation pump chamber (A), which facilitated the internal flow circulation; a packed column chamber (B) filled with sand for biofilm attachment; and a mixing chamber (C) where copper waste was introduced (See). This recirculation system ensured uniform distribution of nutrients and contaminants, promoting stable microbial activity throughout the process.

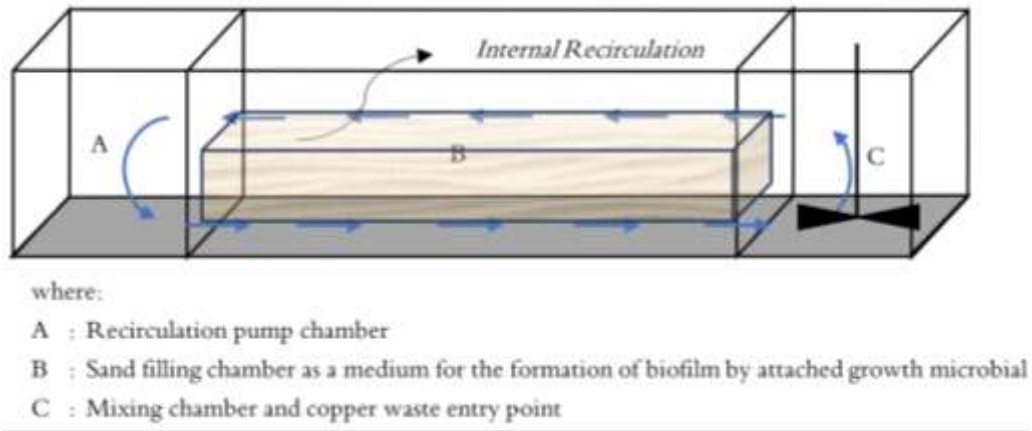

Figure 1. Design of batch process bioreactor with internal recirculation flow using sand filling material as a medium for forming biofilm by attached growth microorganisms

The sand used as a medium is washed clean using aqueous water to remove impurities, then dried and sterilized to avoid contamination. This sand is then put into the bioreactor tank fill column. Clean water is continuously flowed through the sand for several days to allow the formation of biofilms [37]. As an additional nutrient to accelerate microbial growth, 1 tablespoon of granulated sugar is added to the water stream.

The formation of biofilms is characterized by the appearance of a thin layer of mucus on the surface of the sand. This mucus is an extracellular matrix produced by microbes to protect themselves and strengthen adhesion to the sand substrate. This process takes 7 days for the biofilm to reach optimal maturity for use in biosorbation experiments. The 7-day period represents the time required for the adaptation process (lag phase) until the exponential growth phase is reached, which is indicated by the visible formation of biofilm.

2.2. Experimental Setup

The experimental setup was conducted using $CuSO_4$ solution as the source of copper ions. A 1000 mg/L stock solution of Cu was prepared by dissolving 3.92 g of $CuSO_4 \cdot 5H_2O$ in 1 L of deionized water in a volumetric flask. This stock solution was then diluted as needed to obtain working solutions with specific concentrations (10, 40, 100, 200, and 250 mg/L).

The copper solution was introduced into the bioreactor tank through an internal-recirculation system, ensuring continuous circulation throughout the bioreactor. To maintain solution homogeneity, the tank was equipped with a stirrer. Contact time variations were performed by measuring biosorption at time intervals of 0 days, 1 day, 2 days, and 3 days.

The pH of the solution was adjusted using H_2SO_4 to lower the pH or NaOH to increase it, with target values set at pH 6, 7, and 8. After the treatment was completed, the copper solution in the tank was collected for analysis, and the amount of copper ions adsorbed onto the biofilm was calculated based on the difference between the initial and final solution concentrations.

2.3. Analytical Method

2.3.1. Copper Ion (Cu) Concentration Analysis

The concentration of copper ions in the solution was analyzed using Atomic Absorption Spectrophotometry (AAS) at a wavelength of 324.8 nm. Before measurement, the instrument was calibrated using standard Cu solutions with concentrations of 0 mg/L (blank), 25 mg/L, 75 mg/L, 100 mg/L, 150 mg/L, 200 mg/L, and 250 mg/L. The absorbance of the solution was measured, and a calibration graph (standard curve) of concentration versus absorbance was created to determine the Cu concentration in the sample solution (Figure 2).

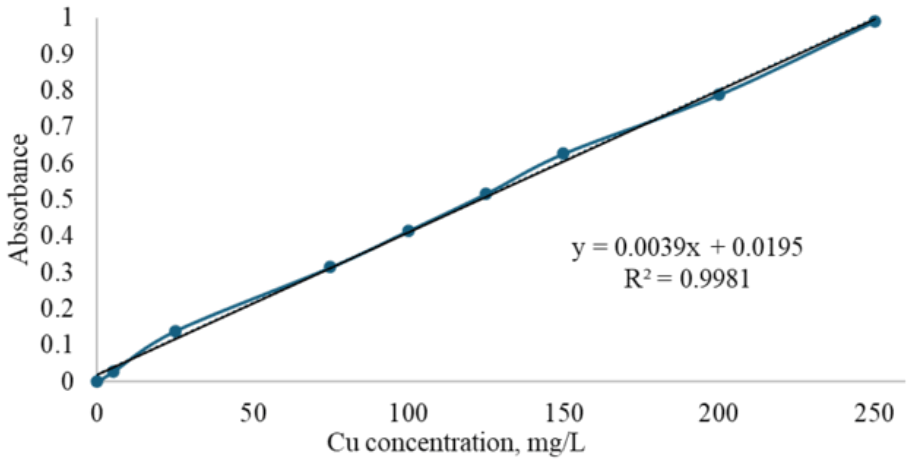

Figure 2 The Standard Curve of Dissolved Copper Concentration versus Absorbance Measured Using Atomic Absorption Spectrophotometry (AAS) at a Wavelength of 324.8 nm.

2.3.2. Chemical Oxygen Demand (COD) Analysis

COD measurement was conducted using the open reflux method in accordance with SNI 06-6989.15-2004. A 10 mL sample solution was mixed with 0.2 g of $HgSO_4$ and 5 mL of 0.25 N $K_2Cr_2O_7$ solution, followed by the addition of 15 mL of concentrated H_2SO_4 solution. The mixture was shaken and refluxed for 2 hours.

After refluxing, the solution was cooled, diluted with deionized water to a total volume of 70 mL, and titrated using 0.1 N Ferrous Ammonium Sulfate (FAS) solution with ferroin indicator. The volume of titrant used was recorded, and the COD value was calculated using the following formula:

$$\text{COD} = \frac{(\text{A} - \text{B}) \times N_{FAS} \times 8000}{\text{V}_\text{S}}$$

where: A is FAS titration volume used for blank titrationo (mL); B is FAS titration volume used for sample titration (mL); N_{FAS} is FAS normality (N); and V_s is Sample volume (mL).

COD reduction performance:

$$\%\ \text{COD removal} = \frac{\text{COD}_{\text{initial}} - \text{COD}_{\text{after}}}{\text{COD}_{\text{initial}}} \times 100\%$$

2.3.3. Biochemical Oxygen Demand (BOD) Analysis

BOD measurements were carried out by the Winkler titration method according to SNI standard 06-6989.14-2004. The solution sample was incubated for 5 days at 20°C in a Winkler bottle, then dissolved oxygen (DO) levels were measured before (DO_0) and after incubation (DO_5). The BOD calculation is carried out using the following formula:

$$\text{BOD} = \frac{\text{P(A-B)N} \times 8000}{\text{V}}$$

where: P is dilution; A is the titrant volume used on DO_0; B is the titrant volume used on DO_5; DO_0 is dissolved oxygen at initial days; DO_5 is dissolved oxygen after 5 days; N is normality of thiosulfate solution; and V is titrized volume.

BOD reduction performance:

$$\%\ \text{BOD removal} = \frac{\text{BOD}_{\text{initial}} - \text{BOD}_{\text{after}}}{\text{BOD}_{\text{initial}}} \times 100\%$$

3. RESULT AND DISCUSSION

3.1. Optimal Contact Time

The biosorption efficiency increased with contact time, reaching an optimal value after 1 day (24 hours). At this point, the absorbance of Cu ions dropped significantly, indicating maximum binding by biofilm functional groups. Prolonged contact time (beyond 1 day) showed no further increase in biosorption, suggesting saturation of active sites on the biofilm.

This finding aligns with kinetic studies of biosorption, where the initial phase is rapid due to abundant binding sites. Over time, as the sites become occupied, the rate of biosorption diminishes until equilibrium is reached. Rapid biosorption within 24 hours demonstrates the practicality of this method for industrial applications, where short processing times are critical (Figure 3).

The biosorption of Cu^{2+} onto the sand-based biofilm was influenced by factors such as pH, initial Cu concentration, and contact time. The observed removal efficiency suggests that biosorption involves a combination of electrostatic attraction, complexation, and ion exchange within the extracellular polymeric substances (EPS) of the biofilm. Future studies should further investigate the adsorption behavior using isotherm models to quantify the adsorption capacity more accurately.

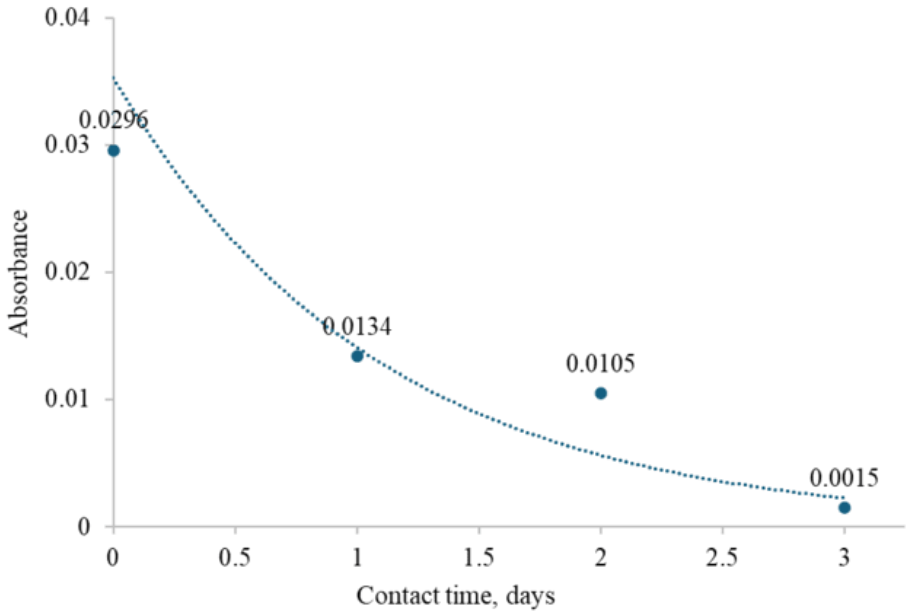

Figure 3. The graph of the relationship between contact time and absorbance for copper ion biosorption at an initial Cu concentration of 4 mg/L shows that Cu ions were reduced to approximately 0.279 mg/L after 3 days (not the optimum time) and only decreased to 2.596 mg/L after 1 day (the optimum time). A 1-day contact time was chosen as the optimum time considering that it provided a significantly reduced Cu concentration within a shorter duration. The coordinate point (1, 0.0134) was considered the closest linear point to the (0,0) coordinate on the graph.

3.2. Influence of Cu Concentration

Initial Cu concentration played a pivotal role in biosorption efficiency. The system achieved maximum efficiency at 100 mg/L, with a biosorption capacity of 39.7 mg/L. Beyond this concentration, the biosorption rate plateaued, likely due to the saturation of binding sites within the EPS. At higher concentrations (200–250 mg/L), the system's performance declined slightly, suggesting competition among Cu ions for limited sites.

The Cu biosorption capacity by bacterial biofilm layers growing on sand media in a batch process bioreactor with internal recirculation, calculated based on the difference between the initial Cu concentration and the Cu concentration after treatment as can be seen in Figure 4 and 5.

$$The\ Reduction\ of\ Cu\ Concentration\ (\Delta C) = [Cu]_{initial} - [Cu]_{after}$$

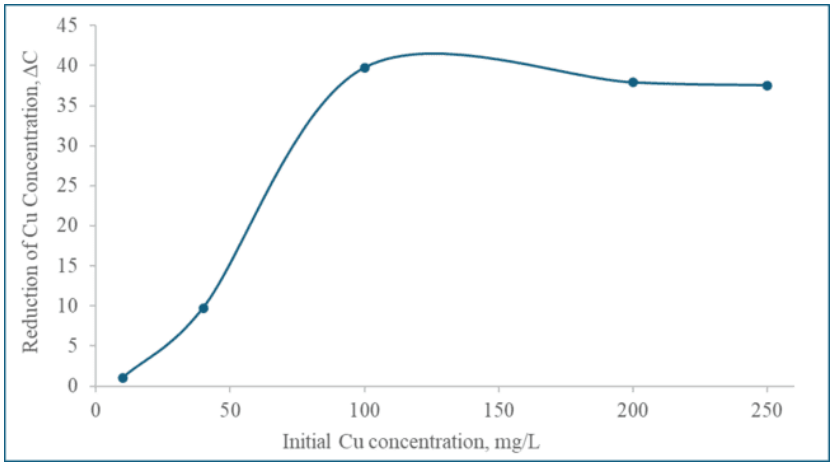

Figure 4. The relationship between the initial dissolved Cu concentration (mg/L) and the Cu biosorption capacity by bacterial biofilm layers growing on sand media in a batch process bioreactor with internal recirculation, expressed based on the reduction in Cu concentration (ΔC) in mg/L after 1 day

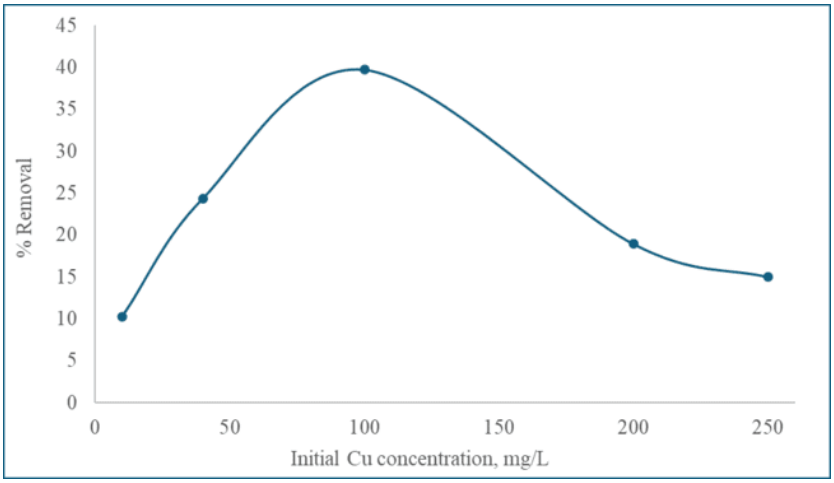

Figure 5. The relationship between the initial dissolved Cu concentration (mg/L) and the Cu biosorption capacity by bacterial biofilm layers growing on sand media in a batch process bioreactor with internal recirculation, expressed based on the reduction in the percentage of Cu removal after 1 day

3.3. Role of pH in Biosorption

The pH significantly influenced the biosorption process. The highest efficiency was observed at pH 8, where Cu ions interacted optimally with negatively charged functional groups in the EPS. At lower pH levels, excess H^+ ions competed with Cu^{2+} for binding sites, reducing biosorption efficiency. Conversely, at pH levels above 8, Cu ions began forming insoluble hydroxides ($Cu(OH)_2$) where its K_{sp} is 2.2×10^{-20}, limiting their availability for biosorption.
These results underscore the importance of maintaining optimal pH conditions to maximize the effectiveness of biofilm-mediated biosorption.

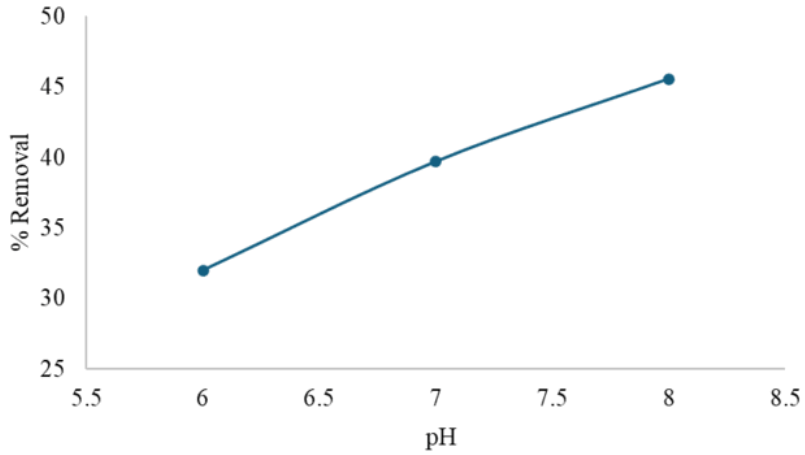

Figure 6. The effect of pH on the efficiency of Cu ion concentration reduction was evaluated at an initial concentration of **100 mg/L**. pH levels above **8** were not evaluated because, at this pH, **Cu precipitates as $Cu(OH)_2$**, making it unavailable for biosorption.

3.4. Water Quality Imporvements

Biosorption not only reduced Cu concentrations but also significantly improved water quality:

- COD: Decreased from 1077.12 mg/L to 247.10 mg/L (77.06%), indicating substantial removal of organic pollutants.
- BOD: Reduced from 372.92 mg/L to 78.62 mg/L (78.92%), suggesting effective biodegradation of organic matter.
- TDS: Declined from 510 mg/L to 357 mg/L (30%), reflecting reduced dissolved solids after treatment.

The reduction in COD and BOD aligns with the biofilm's ability to degrade organic matter, while TDS improvements highlight its effectiveness in capturing inorganic ions. These results demonstrate the dual functionality of biofilms in heavy metal mitigation and organic pollutant removal. Although the levels observed after 1 day of treatment are still below the applicable quality standards set by government regulations in Indonesia, this approach has significant potential for industrial applications. Integration with other processes and the possibility of improving efficiency by replacing the sand medium with alternative materials could further enhance its effectiveness.

Table 1. The performance of metal ion biosorption by microbial biofilm attached to sand media in a batch bioreactor with internal recirculation after 1 day for selected water quality parameters.

Parameters	Test Results			Wastewater Quality Standards*
	Initial	After 1 day	% removal	
COD (mg/L)	1077.12	247.10	77.06	25
BOD (mg/L)	372.92	78.62	78.92	3
TDS (mg/L)	510	357	30	1000
Cu (mg/L)	10	8.97	10.25	0.02
	40	30.25	24.36	0.02
	100	60.28	39.72	0.02
	200	162.13	18.94	0.02
	250	212.49	15.01	0.02

* Referring to Class II Water Quality Standards in Government Regulation of the Republic of Indonesia No. 22 of 2021, Annex VI

Compared to conventional techniques such as activated carbon adsorption and chemical precipitation, biofilm-based biosorption offers several advantages: (1) lower operational costs, (2) minimal secondary waste generation, and (3) simultaneous removal of organic pollutants. While activated carbon has higher adsorption capacities, it requires regeneration, making it less sustainable in the long term.

3.5. Environmental Implications

The significant reduction in COD, BOD, and TDS highlights the broader environmental benefits of using biofilm-based biosorption. By improving water quality, this method supports aquatic ecosystem health and reduces the ecological footprint of industrial effluents. Additionally, the use of sand as a biofilm substrate offers scalability and economic feasibility for widespread implementation.

4. CONCLUSION

This study demonstrates the effectiveness of sand-based biofilms for Cu biosorption and water quality improvement. The optimal conditions—1-day contact time, 100 mg/L Cu concentration, and pH 8—maximize biosorption efficiency while reducing COD, BOD, and TDS. These findings suggest that biofilm-based biosorption is a viable and eco-friendly solution for treating heavy metal-laden wastewater, with significant potential for industrial applications.

These findings suggest that biofilm-based biosorption is a viable and eco-friendly solution for treating heavy metal-laden wastewater. Future research should focus on scaling up the system for industrial applications, optimizing biofilm composition to enhance adsorption efficiency, and integrating this approach with existing wastewater treatment systems for maximum impact. Additionally, testing this method for other heavy metals such as lead (Pb) and cadmium (Cd) would further validate its applicability across various industrial effluents

AUTHOR CONTRIBUTION: **Conceptualization**, Rosna and Zainal Syam Arifin; **methodology**, Ahmad Zaeni; **validation and formal analysis**, Laode Kadidae; **investigation**, Rosna; **resource**, Ahmad Zaeni; **data curation**,

Laode Kadidae; **writing—preparation of original draft**, Rosna; **writing—reviewing and editing**, Zainal Syam Arifin. All authors have read and approved the published version of the manuscript.

FUNDING: -

ACKNOWLEDGMENT: The authors extend their gratitude to Universitas Halu Oleo for providing research facilities. Special thanks to the Laboratory of Biochemistry team for their invaluable assistance during this study.

CONFLICT OF INTEREST: The author declares no conflict of interest.

REFERENCES

1. Ullah, S., Naeem, A., Calkaite, I., Hosney, A., Depar, N., Barcauskaite, K. Zinc (Zn) mitigates copper (Cu) toxicity and retrieves yield and quality of lettuce irrigated with Cu and Zn-contaminated simulated wastewater. *Environmental Science and Pollution Research* **2023**,30(19), 54800-54812. doi:10.1007/s11356-023-26250-8
2. Ankita, Chaudhary, A., Devi, R., Sharma, N. Spectrophotometric Investigations on Some Heavy Metals Cu, Ni, Co, and Mn to Evaluate Their Environmental Toxicity. *Journal of Analytical Chemistry* **2024**, 79(7), 914-922. doi:10.1134/S106193482470028X
3. Garncarek-Musiał, M., Maruszewska, A., Kowalska-Góralska, M., Mijowska, E., Zielinkiewicz, K., Dziewulska, K. Comparative study of influence of Cu, CuO nanoparticles and Cu^{2+} on rainbow trout (*Oncorhynchus mykiss* W.) spermatozoa. *Sci Rep* **2024**, 14(1), 22242. doi:10.1038/s41598-024-72956-1
4. Nagai, T., Kamo, M. Modeling the effects of pH and hardness on the toxicity of zinc, copper, cadmium, and nickel to the freshwater diatom *Navicula pelliculosa*. *Limnology,* **2025**, 1-10. Published online 2025. doi:10.1007/s10201-024-00777-2
5. Wang, Y., Yan, Q., Shi, Y., Long, M. Copper Toxicity in Animals: A Review. *Biol Trace Elem Res* **2024**, Published online 2024. doi:10.1007/s12011-024-04345-8
6. Biedunkova, O., Kuznietsov, P. Dataset on heavy metal pollution assessment in freshwater ecosystems. *Sci Data* **2024**, 11(1), 1241. doi:10.1038/s41597-024-04116-z
7. Seo, C., Lee, J.W., Jeong, J.W., Kim, T.S., Lee, Y., Gang, G., Lee, S.G. Current technologies for heavy metal removal from food and environmental resources. *Food Sci Biotechnol* **2024**, 33(2), 287-295. doi:10.1007/s10068-023-01431-w
8. Li, S., Yan, X., Zhang, M., Sun, Q., Zhu, X. Microbial remediation technology for heavy metal contamination of mine soil. *Chemoecology* **2024**, 34(2), 47-59. doi:10.1007/s00049-024-00399-1
9. Ding, J., Hu, J. Soil heavy metal pollution and health risk assessment around Wangchun Industrial Park, Ningbo, China. *J Soils Sediments* **2024**, 24(7), 2613-2622. doi:10.1007/s11368-024-03806-w
10. Karthikayini, S., Chandrasekaran, A., Narasimhan, C.L. Heavy metal contamination in soils of industrial estates of Tamil Nadu, India and source identification by magnetic susceptibility. *Environ Monit Assess* **2024**, 196(12), 1171. doi:10.1007/s10661-024-13339-x
11. Borgohain, D., Lanong, S., Jaishi, H.P. Heavy metal contamination and health risks in ground water at Byrnihat industrial area: urgent need for remediation and public health safeguards. *Proceedings of the Indian National Science Academy*. 2024, 90(4), 931-942. doi:10.1007/s43538-024-00273-2
12. Tabagari, I., Varazi, T., Dumbadze, N., Kurashvili, M., Pruidze, M., Khatisashvili, G., Karpenko, O., Koretska, N. Biological two-stage treatment technology for mitigating mining-related heavy metal pollution in Georgian rivers. *Discover Water* **2024**, 4(1), 87. doi:10.1007/s43832-024-00128-3
13. John, P.M., Gopinath, A. An Overview of Heavy Metal Pollution in Aquatic Sediments Around the World BT-Contaminated Land and Water: Remediation and Management. In: Alshemmari H, Hashmi MZ, Kavil YN, Shu-hong W, eds. Springer International Publishing, **2024**, 119-129. doi:10.1007/978-3-031-65129-8_9
14. Sarvestani, R.A., Aghasi, M., Niknejad, H. Health risk assessment of trace elements (Pb, Cd, Cu, Fe) in agricultural soil in Kerman City, Southeast of Iran. *Natural Hazards* **2024**, 120(1), 339-367. doi:10.1007/s11069-023-06218-0

15. Boahen, E. Heavy metal contamination in urban roadside vegetables: origins, exposure pathways, and health implications. *Discover Environment* **2024**, 2(1), 145. doi:10.1007/s44274-024-00182-7
16. Jangirh, R., Mondal, A., Yadav, P., Datta, A., Saxena, P., Mandal, T.K. Characterization of Road Dust in Delhi: Heavy Metal Analysis, Health Risks, and Sustainability Implications. *Aerosol Science and Engineering* **2024**, 8(4), 414-425. doi:10.1007/s41810-024-00231-x
17. Samani, M., Ahlawat, Y.K., Golchin, A., Bybordi, A., Sharma, N., Alikhani, H.A. Evaluating seasonal health risks of copper, nickel, and chromium in airborne dust. *Air Qual Atmos Health* **2025**. Published online 2025. doi:10.1007/s11869-025-01704-9
18. Pet, I., Sanad, M.N., Farouz, M., Elfaham, M.M., El-Hussein, A., Abd El-sadek, M.S., Althobiti, R.A., Ioanid, A. Review: Recent Developments in the Implementation of Activated Carbon as Heavy Metal Removal Management. *Water Conservation Science and Engineering* **2024**, 9(2), 62. doi:10.1007/s41101-024-00287-3
19. Twizerimana, P., Wu, Y. Overview of integrated electrocoagulation-adsorption strategies for the removal of heavy metal pollutants from wastewater. *Discover Chemical Engineering* **2024**, 4(1), 14. doi:10.1007/s43938-024-00053-w
20. Machado, A.A., Valiaparampil, J.G. Unlocking the Potential of Algae for Heavy Metal Remediation. *Water Air Soil Pollut.* **2024**, 235(10), 629. doi:10.1007/s11270-024-07436-3
21. Waqeel, J., Khan, S.T. Live Multi-metal Tolerant Bacterial Biofilm on Polyurethane Sponge for Low-cost Bioremediation of Heavy Metal from Small-scale Industry Wastewater. *Int J Environ Res* **2024**, 19(1), 13. doi:10.1007/s41742-024-00674-7
22. Kucuker, M.A. Recovery of Metals from Leach Liquors: Biosorption versus Metal Sulfide Precipitation BT-Biotechnological Innovations in the Mineral-Metal Industry. In: Panda S, Mishra S, Akcil A, Van Hullebusch ED, eds. Springer International Publishing, **2024**, 151-160. doi:10.1007/978-3-031-43625-3_9
23. Dianatdar, F., Etemadifar, Z. Recent Advances Towards Improved Microbial Bioremediation of Heavy Metal Pollution BT-Heavy Metal Remediation: Sustainable Nexus Approach. In: Kumar N, ed. Springer Nature Switzerland, **2024**, 115-138. doi:10.1007/978-3-031-53688-5_6
24. Momin, S.C., Pradhan, R.B., Nath, J., Lalmuanzeli, R., Kar, A., Mehta, S.K. Metal sequestration by Microcystis extracellular polymers: a promising path to greener water treatment. *Environmental Science and Pollution Research* **2024**, 31(7), 11192-11213. doi:10.1007/s11356-023-31755-3
25. Song, X., Yang, A., Hu, X., Ping, N.A., Cao, Y., Zhang, Q. Exploring the role of extracellular polymeric substances in the antimony leaching of tailings by Acidithiobacillus ferrooxidans. *Environmental Science and Pollution Research* **2023**, 30(7), 17695-17708. doi:10.1007/s11356-022-23365-2
26. Li, Z., Zhu, X., Zhang, M., Guo, W., Wu, Q., Wang, J. Study on Adsorption Model and Influencing Factors of Heavy Metal Cu^{2+} Adsorbed by Magnetic Filler Biofilm BT-Proceedings of the 2nd International Conference on Innovative Solutions in Hydropower Engineering and Civil Engineering. In: Wang S, Li J, Hu K, Bao X, eds. Springer Nature Singapore, **2023**, 503-513.
27. Cui, L., Chen, S., Cao, X., Zhang, X., Huang, X., Shibata, T., Yang, Y., Shi, L., Zhao, C., Wang, S., Yang, S. Simultaneous removal of heavy metals and inorganic nitrogen by using the biofilm of Marichromatium gracile YL28. *World J Microbiol Biotechnol* **2024**, 41(1), 14. doi:10.1007/s11274-024-04193-7
28. Radojević, I.D., Jakovljević, V.D., Ostojić, A.M. A mini-review on indigenous microbial biofilm from various wastewater for heavy-metal removal - new trends. *World J Microbiol Biotechnol* **2023**, 39(11),309. doi:10.1007/s11274-023-03762-6
29. Saini, P., Mishra, P. Biofilm Linked Microbial Prospecting of Bioremediation BT-Bioprospecting of Microbial Resources for Agriculture, Environment and Biochemical Industry. In: Saini P, Mishra P, eds. Springer Nature Switzerland, **2024**, 87-108. doi:10.1007/978-3-031-63844-2_5
30. Lian, Z., Yang, Z., Song, W., Sun, M., Gan, Y., Bai, X. Characteristics of EPS from Pseudomonas aeruginosa and Alcaligenes faecalis under Cd(II) stress: changes in chemical components and adsorption performance. *Environmental Science and Pollution Research* **2022**, 29(50), 75883-75895. doi:10.1007/s11356-022-21114-z
31. Marimuthu, S., Rajendran, K. Structural and Functional Characterization of Exopolysaccharide Produced by a Novel Isolate *Bacillus sp.* EPS003. *Appl Biochem Biotechnol* **2023**, 195(7), 4583-4601. doi:10.1007/s12010-023-04368-2

32. Kauser, S., Saeed, A., Farooq, U., Hussain, A. Investigation of extracellular polymeric substances (EPS) from cyanobacteria/blue green algae and their interaction with heavy metal ions: Insights from excitation-emission matrix fluorescence spectroscopy. *Biologia (Bratisl)* **2024**, 79(11), 3461-3473. doi:10.1007/s11756-024-01800-8
33. Shen, L., Yu, X., Zhou, H., Wang, J., Zhao, H., Qiu, G., Chen, Z. Optimization and mechanism studies for the biosorption of rare earth ions by *Yarrowia lipolytica*. *Environmental Science and Pollution Research* **2024**, 31(39), 52118-52131. doi:10.1007/s11356-024-34660-5
34. Kurniawan, A., Fukuda, Y. Analysis of the electric charge properties of biofilm for the development of biofilm matrices as biosorbents for water pollutant. *Energy Ecol Environ* **2023**, 8(1), 62-68. doi:10.1007/s40974-022-00253-6
35. Wu, C., Jun, W.Y., Wei, Y.S., Li, F. Enhancing cadmium biosorption capacity in *E. coli* through heterologous expression of metal-chelating proteins: Insights into bioremediation potential and mechanisms. *J Cent South Univ* **2024**, 31(4), 1265-1275. doi:10.1007/s11771-024-5625-4
36. Ting, A.S.Y., Tiew, K.H., Song, K.P. Metal biosorption by packed-bed column of exopolymeric substance modified alginate beads for treatment of multi-metal systems. *International Journal of Environmental Science and Technology* **2024**. Published online 2024. doi:10.1007/s13762-024-06224-0
37. Adegoke, A.E., Abel, O.M., Ikechukwuka, E.M., Opeyemi, A.O.M., Nifemi, A.O. Microbial Biofilm Reactor for Sustainable Wastewater Treatment BT-Green Technologies for Industrial Waste Remediation. In: Mathuriya AS, Pandit S, Singh NK, eds. Springer Nature Switzerland, **2023**, 285-316. doi:10.1007/978-3-031-46858-2_14