Advances in Tropical Biodiversity and Environmental Sciences
9(3): 187-193, October 2025
e-ISSN:2622-0628
DOI: 10.24843/ATBES.2025.v09.i03.p07
Available online at: https://ejournal1.unud.ac.id/index.php/atbes/article/view/2018

Effectiveness Test of Immobilization of *Aspergillus niger* Fungi with Different Biomass on Hexavalent Chromium (Cr (VI)) in Water

Anisa Fitriawati*, Ima Yudha Perwira, and Devi Ulinuha

Study Program of Aquatic Resources Management, Faculty of Marine and Fisheries,
Udayana University, Bali, Indonesia
Jl. Raya Kampus Unud, Jimbaran, Kuta Selatan, Kabupaten Badung, Bali 80361
*Corresponding author: anisa.fitriawati@student.unud.ac.id

Abstract. Heavy metal contamination with Cr(VI) in aquatic environments is a serious concern due to its high toxicity and mobility. This research aimed to evaluate the effectiveness of immobilized Aspergillus niger in sodium alginate with different biomass concentrations (0 g, 0.1 g, 0.3 g, and 0.5 g) in reducing Cr(VI) content in water. The research was conducted from March to May 2025 using a quantitative experimental method with a Completely Randomized Design (RAL), consisting of four treatments and three replications. The bioremediation process lasted 5 days, and Cr(VI) concentration was measured using a UV-Vis spectrophotometer at 540 nm, with the data analyzed in Microsoft Excel. The results indicated that each biomass treatment exhibited varying levels of Cr(VI) removal. The treatments T3, T1, T2, and T0 (control) showed the highest to lowest effectiveness, with values of 39.4%, 32.4%, 30.7%, and 28.0%, respectively. The rate of Cr(VI) reduction increased with increasing fungal biomass, but not in a straight line across all treatments. The metal removal process occurred through biosorption and bioaccumulation mechanisms, influenced by factors such as initial metal concentration, particle size, and the availability of functional groups on the fungal cell wall and alginate matrix. This study demonstrates that immobilized Aspergillus niger can reduce Cr(VI) content in water, although its effectiveness is not yet optimal.

Keywords: *Aspergillus niger*; bioremediation; Cr(VI); immobilization

I. INTRODUCTION

Heavy metal pollution in aquatic environments has become a recurring environmental issue in Indonesia over the past few decades. The presence of these heavy metals is often linked to industrial activities that directly discharge untreated waste into water bodies. One of the heavy metals commonly identified in industrial processes is chromium [1]. Chromium is widely used in the leather tanning industry, where only about 60–70% of the chromium is effectively absorbed during processing, while the remaining 30–40% is released into the aquatic environment as waste [2]. Once discharged into water, chromium may oxidize to form hexavalent chromium (Cr^{6+}), which is more mobile and highly toxic. This compound can interfere with the metabolic processes of aquatic organisms, leading to degradation, hemorrhage, and vacuolar damage [3].

Various conventional methods have been applied to address Cr(VI) contamination; however, these approaches often require intensive maintenance and incur high operational costs. An alternative, environmentally friendly method is bioremediation, which uses microorganisms such as macroalgae, bacteria, and fungi. Fungi in particular are potential biocatalysts due to their ability to survive across a wide range of environmental conditions [4]. One of the fungal species commonly used as a biocatalyst, originating from industrial fermentation residues, is *Aspergillus niger*. Although this species is recognized for its ability to remediate chromium in aquatic environments, its relatively small size and low density make it difficult to place at specific contamination points. Therefore, cell

immobilization techniques are needed to enhance its stability and usability [5].

Immobilization is a method for entrapping cells within a polymer matrix through encapsulation, which uses sodium alginate and Ca^{2+} ions to form a stable gel (bead) structure [6]. The effectiveness of immobilization is influenced by several factors, including the type and amount of fungal biomass used, whether living or inactive [7]. Due to the limited studies on optimizing biomass levels in immobilization systems, this research is essential for evaluating the effectiveness of immobilized Aspergillus niger at varying biomass concentrations in reducing Cr(VI) levels in water. The findings are expected to contribute to the development of sustainable wastewater treatment systems.

II. METHODS

A. Time and Location

This research was conducted over three months, from March to May 2025. The entire series of activities was carried out at the Fisheries Science Laboratory, Faculty of Marine Affairs and Fisheries, Udayana University.

B. Tools and Materials

The main tools used to support the research process include measuring cups, test tubes, Erlenmeyer flasks, UV-Vis spectrophotometers, Laminar Air Flow, micropipette, 220 mL bottles, and an analytical balance. The main material in this study consists of isolated Aspergillus niger, sodium alginate, aqueous, $CaCl_2$, standard solution Cr(VI) ($K_2Cr_2O_7$), and Chromium VI HR Reagent Hanna HI 723-25.

C. Research Methods

This research is classified as quantitative research using an experimental method with a Complete Randomized Design (RAL), consisting of four treatments (fungi biomass 0 g, 0.1 g, 0.3 g, and 0.5 g) with three replicates, for a total of 12 experimental units. Quantitative research is a type of research that analyzes numerical data, aiming to provide an accurate and systematic picture of the phenomenon under study [8]. This research was carried out by testing the influence of independent variables on dependent variables using a controlled experimental design, thereby allowing the results to be accounted for. This type of experimental design series is commonly referred to as Complete Random Design (RAL) [9].

1. Fungal culture rejuvenation

The pure isolate of *Aspergillus niger* was rejuvenated using PDA (Potato Dextrose Agar) media sterilized at 121°C for 15 minutes. After inoculation, the media is incubated for 6 days at 35–40°C to obtain active biomass [10].

2. Preparation of stock solution and standard curve of Cr(VI) 100 ppm

The manufacture of the parent solution and the Cr(VI) standard curve is carried out as a reference for the measurement of Cr(VI) levels in the test sample. A 100 ppm parent solution is made in accordance with SNI 6989.71:2009 by dissolving 0,0283 g of $K_2Cr_2O_7$ in 100 mL of sterile aqueducts, then diluted into a solution with a concentration of 1 ppm and divided into 0,0 series; 0,1; 0,2; 0,5; and 0.8 ppm. Each solution was diluted to 1 mL, then 0.015 g of Chromium VI HR Reagent HI 723-25 was added, and its absorbance was measured at 540 nm using a UV-Vis spectrophotometer [11].

The measurement results are analyzed as a linear standard curve, with the x-axis converted from absorbance to concentration. A good R^2 value is $0.9 \leq R^2 \leq 1$, indicating a strong correlation according to the Lambert-Beer law [12]. The linear equation of the curve serves as the basis for measuring Cr(VI) concentration in subsequent studies.

3. Mobilized fungi manufacturing

Mobilized fungi are prepared with a 4% sodium alginate solution mixed with fungal biomass, according to the treatment dose (biomass 0 g, 0.1 g, 0.3 g, and 0.5 g). The mixture is dripped into a 0.2 M $CaCl_2$ solution using a syringe to form Ca-alginate beads (granules). The beads were then washed with sodium chloride, filtered, and weighed, up to 10 g per treatment or repetition. The beads are stored in a refrigerator at 4°C before use on a research scale.

4. Chromium Reduction Capacity Test by Mobilized Fungi

This study uses a Cr(VI) wastewater treatment system by soaking *Beads* in a bottle. The artificial wastewater used in this research contained 100 ppm Cr(VI) in 100 mL. The concentration used is determined based on the general concentration observed in textile industrial waste production, then multiplied by 2 to account for an anticipated increase in the Cr(VI) content of waste discharged into the environment. The concentration of Cr(VI) waste was measured to assess the effectiveness of the reduction by monitoring the rate of decline over the next 4 days.

5. Measurement of Cr(VI) content in water

A 1 mL water sample was taken from each unit. Treatment and repetition using a micropipette were performed, and the test tube was filled with 9 mL of sterile

aqueducts. Re-encryption was carried out using the same procedure, and then 0,5 mL of the sample was transferred into a tube with the addition of 0,5 mL of sterile aqueducts. Each sample is homogenized and mixed with Chromium VI HR Reagent Hanna HI 723-25 up to 0015 g as a specific color reagent. Each mixture is waited for 5 minutes until the color is stable, then measured using the UV-Vis spectrophotometer. The concentration of Cr(VI) in the sample is calculated based on the standard curve that has been created from the standard solution. The procedure was repeated daily for 4 days of the test period to monitor changes in Cr(VI) levels in the bioremediation system, produced by the fungus in the beads.

D. Data analysis

The calculation of the rate of reduction of Cr(VI) levels by fungi is carried out by calculating the concentration of absorbed metals using the Langmuir method with the following equation [13]:

$$Cs = Co - Ct$$

The level of effectiveness of chromium heavy metal removal by fungi was determined by looking for the value of the percentage reduction in concentration from the beginning to the end of the study, the equation formula used [14]:

$$R\ (\%) = \frac{Co - Ct}{Co} \text{ x } 100\%$$

Information:

Cs = Reduction rate/absorbed Cr rate (mg/L)

R = Cr reduction effectiveness (%)

Co = Initial concentration (mg/L)

Ct = Final concentration (mg/L)

All data are analyzed descriptively, quantitatively, and presented in the form of graphs and tables using Microsoft Excel. Quantitative descriptive analysis is used to describe data collected without the intention of making general conclusions or generalizations [15].

III. RESULTS AND DISCUSSION

A. *Effectiveness of Hexavalent Chromium Elimination*

The data shown in Table 1 shows that the addition of immobilized *Aspergillus niger* biomass generally increases the effectiveness of chromium removal. The treatment with the highest biomass, T3 (0,5 g biomass), showed the highest elimination effectiveness of 39%. The T1 treatment (0,1 g biomass) showed 32,4% elimination effectiveness, while T2 (0,3 g biomass) showed a slight decrease to 30,7%. The control treatment T0 (0 g biomass) showed an elimination effectiveness of 28,0%. The decrease in chromium concentration from each celliner treatment with the increase in the amount of biomass, judging from the difference in the increase in allowance by 4,3% from (T1) treatment compared to the control treatment (T0), indicates that the small amount of biomass has begun to contribute to the removal of heavy metals. In contrast to (T2) treatment, the level of preparation was not linear with the addition of the amount of biomass, while the difference in the increase in treatment 3 allowance was 11,4% higher than the control and 7,0% higher than treatment 2

TABLE 1.
PERCENTAGE DECREASE IN HEXAVALENT CHROMIUM CONTENT WITH VARIOUS TREATMENTS

Treatment (grams)	Initial Concentration (mg/L)	Final Concentration (mg/L)	Effectiveness of Elimination (%)
T0 (0)	96,6 ± 4,0	69,5 ± 4,3	28,0
T1 (0,1)	101,1 ± 5,7	68,4 ± 7,7	32,4
T2 (0,3)	98,4 ± 5,2	68,1 ± 3,3	30,7
T3 (0,5)	99,5 ± 1,5	60,30 ± 7,2	39,4

Aspergillus niger fungus has the potential to reduce Cr(VI) content in water. Although the elimination effectiveness has not reached 50%, this study has demonstrated that Aspergillus niger can be used in wastewater treatment under actual conditions. The previous method at laboratory scale achieved a Cr elimination effectiveness rate of more than 90%, but only in small- to medium-sized systems under optimally supported environmental conditions. It is necessary to contain microbial cells in a limited space to enable a catalytic process to be carried out repeatedly or continuously through immobilization. Immobilization in this research uses the encapsulation technique to hold cells in a semipermeable alginate buffer matrix, allowing nutrients and metabolic products to diffuse.

Polymers extracted from seaweed or alginate can form microspheres when combined with microorganisms, and have been shown to be effective in decreasing Cr(VI) when used with *Aspergillus niger* [16]. T1 and T3 treatments showed Cr(VI) elimination rates of 32,4% and 39,4%,

respectively, compared to controls (T0), demonstrating that immobilization can expand the active surface and strengthen interactions with Cr(VI) [17]. Even in the T0 treatment, the effectiveness level of 28,0% is thought to result from the adsorption capacity of the alginate matrix. Increased biomass does not necessarily increase absorption effectiveness, as in T2, which obtained a yield of 30,7%, slightly below P1, which is suspected to be due to inhibition of ion diffusion to cells by several factors [18].

Although the decline in Cr(VI) content in the water between the treatment and no-treatment conditions was not large, in general, the addition of mobilized Aspergillus niger biomass decreased Cr(VI) content in the water. *Aspergillus niger,* together with a sodium alginate mixture, contains functional groups such as carboxyls, hydroxyls, and amines that play an active role in metal ion binding [19]. Cr uptake occurs through chemical-physical adsorption, involving ion exchange and interaction with the cell wall [20]. Although *Aspergillus niger* is effective for heavy metal Cr, nutrient limitations can inhibit the process of bioremediation mechanisms, especially in metals such as Cr(VI), which are not essential, in contrast to Zn or Hg, which are more easily absorbed due to ionic suitability and biological requirements for *Aspergillus niger* [21]-[22].

B. Rate of Decrease in Hexavalent Chromium Content

In general, the results of the 4-day reduction test for the overall biomass treatment showed that each treatment affected the final Cr(VI) concentration in the water during the study. The rate of decrease in chromium content in water over the 4 days of the research varied (Table 2). The initial chromium concentration in water from each treatment ranged from 96,6 mg/L to 101,1 mg/L. After four days, the chromium concentration decreased to 60,3-69,5 mg/L. Treatment with 0,5 g biomass (T3) showed the lowest final concentration (60,3 mg/L), indicating the greatest rate of decline among the treatments.

The rate of decline in Cr(VI) content with increasing concentration absorbed is shown in Table 4.3. Decrease in T0 concentration, which was 96,6 mg/L to 69,5 mg/L, with an average amount of absorbed concentration of about 6,8 mg/L. Decrease in T2 concentration, which was originally 101,1 mg/L to 68,4 mg/L, with a total absorption concentration of about 8,2 mg/L. Decrease in T3 concentration, which was originally 98,4 mg/L to 68,1 mg/L, with a total absorption concentration of about 7,6 mg/L. The highest absorbed concentration in the T3 treatment was around 9,8 mg/L, with an initial concentration of 99,5-60,3 mg/L.

The graph showed a decrease in Cr(VI) concentration in all treatments during 4 days of incubation. The higher the treatment dose, the greater the rate of decrease in Cr(VI) concentration tends to be. The T3 treatment (0,5 g) yields the highest reduction in Cr(VI) content, especially from day 2 to day 4. A visualization of the rate of decline between treatments is shown in Figure 1.

The rate of decline in Cr(VI) content with increasing absorbed concentration is shown in Table 3. Decrease in T0 concentration, which was all 96,6 mg/L to 69,5 mg/L, with the average total absorbed concentration was 6,8 mg/L. Decrease in T1 concentration, which was originally 101,1 mg/L to 68,4 mg/L, with the average total absorbed concentration was 8,2 mg/L. Decrease in T2 concentration, which was originally 98,4 mg/L, to 68,1 mg/L, with an average total absorbed concentration of 7,6 mg/L. The highest average absorbed concentration was found in the T3 treatment, which was 9,8 mg/L, with an initial concentration of 99,5 mg/L to 60,3 mg/L.

The rate of decline in Cr(VI) concentrations over up to 5 days, particularly at high biomass doses, indicates that, in addition to rapid bioabsorption at the cell surface, there may also be a slower, dependent bioaccumulation process linked to the cell's metabolic activity. Although biosorption generally reaches equilibrium in a short period of time, from minutes to hours, long-term decline indicates slower mechanism involvement. Bioaccumulation of Cr(VI) ions that depend on the metabolism of living cells or biotransformation of valence changes in Cr(VI) ions contributes significantly to the decline in Cr content, so that it lasts more slowly [23]-[24].

TABLE 2.
RATE OF DECREASE IN HEXAVALENT CHROMIUM CONTENT FOR 4-DAYS

Treatment (grams)	Day				
	0	1st	2nd	3rd	4th
T0 (0)	96,6 ± 4,0	82,3 ± 5,0	78,6 ± 4,2	77,3 ± 4,4	69,5±4,3
T1 (0,1)	101,1 ± 5,7	85,3 ± 6,0	81,0 ± 4,6	77,2 ± 3,3	68,4±7,7
T2 (0,3)	98,4 ± 5,2	83,4 ± 5,3	79,4 ± 4,2	72,0 ± 2,4	68,1±3,3
T3 (0,5)	99,5 ± 1,5	86,9 ± 3,3	78,6 ± 2,1	69,7 ± 5,2	60,3±7,2

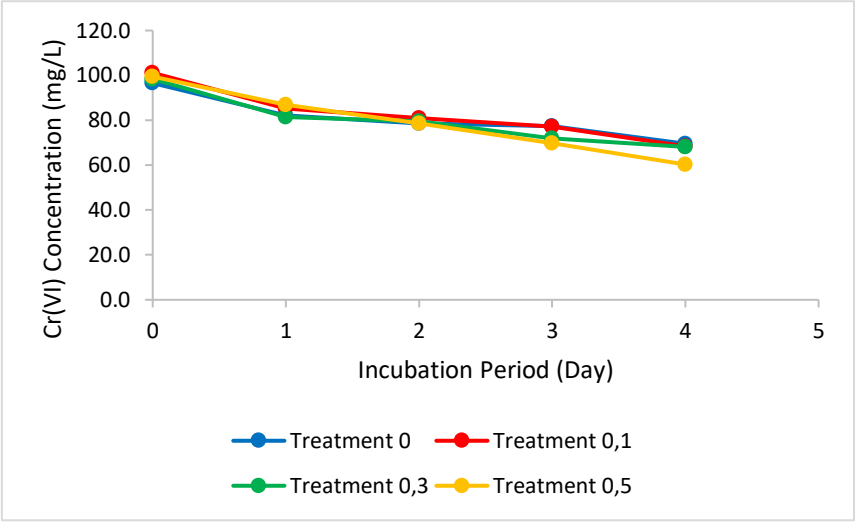

Figure 1. Graph of the rate of decrease in hexavalent chromium content

TABLE 3.
TOTAL CR(VI) CONCENTRATION ABSORBED FOR 4 DAYS

Treatment (grams)	Day					Average Reduction Rate (mg/L)
	0	1st	2nd	3rd	4th	
T0 (0)	96,6	82,3	78,6	77,3	69,5	
		14,4	3,7	1,2	7,8	6,8
T1 (0,1)	101,1	85,3	81,0	77,2	68,4	
		15,9	4,2	3,8	8,8	8,2
T2 (0,3)	98,4	83,4	79,4	72,0	68,1	
		15,0	4,0	7,4	3,9	7,6
T3 (0,5)	99,5	86,9	78,6	69,7	60,3	
		12,5	8,4	8,8	9,4	9,8

The variation in the reduction rate indicates the presence of a biosorption mechanism involving two processes: adsorption and absorption. Adsorption occurs passively when Cr(VI) ions interact with functional groups on the cell surface, including via an ion-exchange process that allows extracellular accumulation of metals [25]. Meanwhile, absorption occurs actively when the negatively charged cell wall binds to chromate ions ($CrO7^{2-}$), which are then transported across the cell membrane, allowing them to accumulate and be metabolized intracellularly. The accumulated Cr(VI) ions subsequently settle and bind to the functional group in the mobilized *Aspergillus niger* biomass in the alginate matrix [26].

The successful absorption of Cr(VI) is influenced by various factors, including biomass dose, ionic strength, initial concentration, and particle size. High doses provide more active sites but can lower specific efficiency due to particle overlap, while high ionic strength can inhibit adsorption by competing with other ions [27]. High concentrations increase total absorption capacity, but relative efficiency can decrease when the number of active sites is limited, and small particle sizes expand the adsorption surface but risk interparticle blockage [28].

IV. CONCLUSION

Based on the results of a study on the effectiveness test of *Aspergillus niger* fungi mobilized using alginate with variations in the amount of biomass to decrease in hexavalent chromium (Cr(VI)) levels in water, it can be concluded that:

1. The difference in the value of the reduction result between administration or without treatment was not much different and had not reached a 50% result, although the addition of *mobilized Aspergillus niger* biomass had contributed to a decrease in the Cr(VI) content in the water in the T3 treatment by 39,4%, so it could not be said to be optimal. It is caused by the diffusion of metal ions in the matrix, the mismatch between the Cr(VI) charge and the fungal functional

groups, and nutrient limitations that inhibit metabolic processes.

2. There was a decrease in Cr(VI) concentration in all treatments during the 4-day incubation period, with the highest amount of absorbed concentration shown in the T3 treatment reaching 9,8 mg/L. The rate of concentration decline during the bioremediation process was influenced by various factors, including biomass dose, increased ionic strength, initial concentration, and particle size.

REFERENCES

[1] Aminatun, T., Rakhmawati, A., Budiasih, K.S., Marfuatun, Rijal, B.S., Amin, A.N., Arifin, D.M.N., dan Putri, A.S. 2024. Identifikasi Logam Berat Kromium di Tiga Sungai yang Melintasi Kota Yogyakarta dan Potensi Fitoremidiasinya. *Jurnal Ilmu Lingkungan,* 22(3): 620-630. https://doi.org/10.14710/jil.22.3.620-631

[2] Maryudi, Rahayu, A., Syauqi, R., dan Islami, M. K. 2021. Teknologi Pengolahan Kandungan Kromium dalam Limbah Penyamakan Kulit Menggunakan Proses Adsorpsi: Review. *Jurnal Teknik Kimia dan Lingkungan,* 5(1): 90-99. 10.33795/jtkl.v5i1.207

[3] Kurniawan, A. and Mustikasari, D., 2019. Review: Mekanisme Akumulasi Logam Berat di Ekosistem Pascatambah Timah. *Jurnal Ilmu Lingkungan ,* 17(3): 408-415. 10.14710/jil.17.3.408-415

[4] Rabab, A.M., Asmaa, S., Asmaa, H.M., and Shereen, A.S. 2025. Adaptability assessment of Aspergillus niger and Aspergillus terreus isolated from long-term municipal/industrial effluent-irrigated soils to cadmium stress. *Microbiology,* 297: 1-16.

[5] Chug, R., Gour, V.S., Mathur, S. and Khotari, S. L., 2016. Optimization of Production Using Azotobacter beijreinckii and Bacillus subtilis and Its Application in Chromium (VI) Removal. *Bioresource Technology,* Volume 214: 604-608. 10.1016/j.bbrep.2021.100972

[6] Nurmalasari, R., Pratiwi, S.W., Pinarti, I., dan Abiyasa, G. 2022. Penentuan Kapasitas Adsorpsi Logam Kromium pada Mikrokapsul Ca-ALGINAT-EDTA. *Staba,* 6(2): 1-5.

[7] Ren, B., Zhang, Q., Zhang, X., and Zhao, L., Li, H. 2018. Biosorption of Cr (VI) from aqueous solution using. *Royal Society of Chemistry,* 8: 38157-38165

[8] Sulistyawati, W.W. dan Trinuryono, S. 2022. Analisis (Deskriptif Kuantitatif) Motivasi Belajar Siswa dengan Model Blended Learning di Masa Pandemi Covid19. *Jurnal Matematika dan Pendidikan Matematika,* 13(1): 67-72.

[9] Rahmawati, A. S. dan Erina, R. 2020. Rancangan Acak Lengkap (RAL) dengan UJi Anova Dua Jalur. *Optika: Jurnal Pendidikan Fisika,* 4(1): 54-62. oai:ojs2.e-journal.uniflor.ac.id:article/333

[10] Nuban, A.A., 2021. Biosorpsi Timbal (Pb) pada Larutan Menggunakan Aspergillus niger Amobil: Teknologi Hijau untuk Penghilangan Logam Berat. *The Indonesian Green Technology Journal,* 10(1): 21-27. 10.21776/ub.igtj.2021.010.01.03

[11] Andini, A., 2017. Analisa Kadar Kromium VI [Cr (VI)] Air di Kecamatan Tanggulangin, Sidoarjo. *Jurnal Sain Health,* 1(2): 55-58. 10.51804/jsh.v1i2.105.55-58

[12] Nisah, K., dan Nadhifa, H. 2020. Analisis Kadar Logam Fe dan Mn pada Air Minum dalam Kemasan (AMDK) dengan Metode Spektrofotometri Serapan Atom. *Ar-Raniry Chemistry Journal,* 2(1): 6-12.

[13] Ren, B., Zhang, Q., Zhang, X., Zhao, L., Li, H., 2018. Biosorption of Cr (VI) from aqueous solution using. *Royal Society of Chemistry,* 8: 38157-38165. 10.1039/c8ra07084a

[14] Dini, L., Kusumadewi, R.A. and Hadisoebroto, R., 2023. Penyisihan Logam Berat Krom (Cr) dan Zat Warna Menggunakan Adsorben Kulit Pisang Kepok dalam Air Limbah Batik. *Jurnal Reka Lingkungan,* 11(1): 37-48. https://doi.org/10.26760/rekalingkungan.v11i1.37-48

[15] Sugiyono., 2018. *Metode Penelitian Kuantitatif, Kualitatif, dan R&D*. Bandung: Alfabeta. 334 hlm.

[16] Lakshmi, S., Suvedha, K., Sruthi, R., Lavanya, J., Varjani, S., 2020. Hexavalent Chromium Sequestration From Electronic Waste By Biomass Of Aspergillus Carbonarius. *Bioegineered,* 11(1): 708-717. https://doi.org/10.1080/21655979.2020.178082

[17] Ali, E.A.M., Sayed, Mohsen A., Abdel-Rahman, T. M.A., Hussein, R., 2021. Fungal Remidiation of Cd (II) from Wastewater Using Immobilization Techniques. *Royal Society of Chemistry Advances*, 11: 4853-4863. 10.1039/d0ra08578b

[18] Maghraby, Y.R., El-Shabasy, R.M., Ibrahim, A.H. and Azzazy, H.M.E.S., 2023. Enzym Immobilization Technologie and Industrial Applications. *Acs Omega,* 8: 5184-5196. 10.1021/acsomega.2c07560

[19] Escudero, C., Fiol, N., Villaescusa, I., and Bollinger, J.-C., 2017. Effect of Chromium Speciation on Its Sorpstion Mechanism onto Grape Stalks Entrapped into Alginate Beads. *Arabian Journal of Chemistry*, 10: 1-11. https://doi.org/10.1016/j.arabjc.2013.03.011

[20] Li, X., Chao, Z., Li, H., Ji, J., Sun, X., Chen, Y., Li, Z., Li, Z., Li, C., Yao, J., Xiang, L., 2024. Heavy Metal Immobilization by Phosphate-Solubilizing Fungus and Phosphogypsum Under the Co-Existence

of Pb(II) and Cd(II). *Agronomy,* 15(7): 1-12. https://doi.org/10.3390/agronomy15071632

[21] Rodriguez, I.A., Gonzalez, J.F.C., Perez, A.S.R., Oviedo, J. T., Juarez, V.M.M., 2018. Bioremoval of Different Heavy Metals by Resistant Fungal Strain *Aspergillus niger*. *Bioinorganic Chemistry and Applications,* 1-7. https://doi.org/10.1155/2018/3457196

[22] Sazanova, K.V., Zelenskaya, M.S., Korneev, A.V., Elena, BV., Vlasov, D.Y., Frank-Kamenetskaya, O.V., 2025. Effect of Fungal Metabolism on Zn Minerals Formation The Case of *Aspergillus niger* and Penicillium chrysogenum. *Crystals,* 15(2): 1-19. 10.3390/cryst15020118

[23] Priyanka., Neelam, S., Bajpai, B., 2018. Effect of Metal Training on *Aspergillus niger* (Lin.) and its Biosorption Capacity. *International Journal of Tropical Agriculture*, 36(3): 827-831. https://doi.org/10.4172/1948-5948

[24] Tang, H., Xiang, G., Xio, W., Yang, Z., Zhao, B., 2024. Microbial Mediated Remediation of Heavy Metals Toxicity: Mechanisms and Future Prospects, *Frontiers in Plans Science*, 15: 1-23. https://doi.org/10.3389/fpls.2024.1420408

[25] Ayele, A., Haile, S., Alemu, D., Khamaraj, H. 2021. Comparative Untilization of Dead and Live Fungal Biomass for the Removal of Heavy Metal: A Concise Review. *The Scientific World Journal*, 2021(1): 1-10. 10.1155/2021/5588111

[26] Xu, H., Hao, Rui-Xia., Xu, Xi-Yang., Ding, Y., Lu, An-Hui., Li, Yin-Huang., 2020. Removal of Hexavalent Chromium by Aspergillus niger Through Reduction and Accumulation. *Geomicrobiology Journal,* 38(1): 20-28. 10.1080/01490451.2020.1807659

[27] Mangwandi, C., Kurniawan, T.A. and Albadarin, A. B., 2020. Comparative Biosorption of Chromium (VI) Using Chemically Modified Date Pits (CM-DP) and Olive Stone (CM-OS): Kinetics, Isotherms and Influence of Co-Existing Ions. *Chemical Engineering Research and Design*, 156: 251-262. https://doi.org/10.1016/j.cherd.2020.01.034

[28] Lindholm-Lehto, P. C., 2019. Biosorption of Heavy Metals by Legnocellulosic Biomass and Chemical Analysis. *Bioresources,* 14(2): 4952-4995. 10.15376/biores.14.2.Lindholm-Lehto