Biosorption of copper by *Alcaligenes faecalis* and *Delftia tsuruhatensis* isolated from wastewater

Yumna Husna Nisaa[1,2*], *Anto Budihardjo*[2], *Sri Pujiyanto*[2]

[1]Biolology Graduate Program, Faculty of Sains and Mathematics, Diponegoro University
[2]Department of Biology, Faculty of Sains and Mathematics, Diponegoro University

Abstract: Biosorption techniques can remove Cu. Copper-resistant bacteria isolated from Cu-contaminated environments. This study test the ability of *Alcaligenes faecalis* and *Delftia tsuruhatensis* to absorb Cu. The concentrations of copper used for the biosorption tests were 50, 100, and 150 mg/L in the nutrient broth media. The concentration of Cu in the nutrient broth media after the biosorption test was measured using an atomic absorption spectrophotometer. *A Scanning Electron Microscope* (SEM) was used to confirm metal biosorption on bacterial cell surfaces. The ability of *Alcaligenes faecalis* to remove copper was 34%, and *Delftia tsuruhatensis* was 45% at the highest concentration. The results showed that *Delftia tsuruhatensis* has the highest capacity for copper biosorption. The results of the condition of *Delftia tsuruhatensis*, with copper metal (Cu), showed that the bacterial cells had an irregular cell shape, and there was a build-up of heavy metal molecules.

1 Introduction

Water pollution can cause public health problems, reduce agricultural productivity, and damage natural biology [1]. The Indonesian Central Statistics Agency reported that water pollution will affected 6.160 villages in 2021. Heavy metals are hazardous pollutants in the environment because they can be accumulate in organisms that consume contaminated food in the food chain [2]. Copper is an example of toxic heavy metal at high concentrations in living organisms at high concentrations [3]. It can cause liver and kidney damage, anemia, and irritation of the digestive tract [4]. Bioremediation could be a useful bacterial technique for reducing or recovering heavy metal to minimize their harmful forms [5].

The advantages of using the bioremediation process to reduce heavy metal waste are cost-effectiveness and environmental friendliness. This bacterial utilization technique is likely able to degrade pollutants and convert them into harmless properties through a redox process

* Corresponding author: yumnahusnanisaa06@gmail.com

[5]. Bacteria are biosorbents that can help improve polluted environments because these cells can grow under controlled conditions and survive in polluted environments [6]. The mechanism of heavy metal removal occurs through metabolic or physicochemical processes by the biomass of active or inactive bacterial cells [7], [8]. It occurs in bacterial cell walls [9]. Bacterial cells must adapt to changes in physical, chemical, and bioreactor configurations during metal remediation [10].

The bacteria used in the bioremediation process were isolated from an environment polluted with heavy metals. In this study, the bacteria were isolated from Sayung Demak ditch surrounded by industrial surroundings, including electronics, pet food, and textile companies. Recent research has shown that the copper content in the Gonjol River is 0.05 mg/L - 0.42 mg/L [11] ,which exceeds the water quality threshold 0.02 mg/L. A previous study showed that nine bacteria isolated from domestic liquid waste were resistant to Cu at a concentration of 25 mg/L [12]. Bacteria isolated from polluted environments have been used for the biosorption of copper metal, such as bacteria from the Cisadane River, namely *Pantoea agglomerans* (1.42 mg/g), *Klebsiella penumoniae* (1.55 mg/g), and *Shigella flexneri* (0.92 mg/g) [13]. Therefore, this research to show the effectiveness *Alcaligenes faecalis* and *Delftia tsuruhatensis* of the Sayung Demak ditch as biosorption agents for copper metals.

2 Materials and methods

2.1 Isolation and identification of bacteria

Bacteria were isolated from the wastewater samples using a serial dilution technique (dilution 10^{-1} to 10^{-4}) [14]. Next, 0.1 ml of each of these dilutions was inoculated using the spread plate technique on the nutrient agar media, which had been supplemented with 5 mg/L of Cu metals and incubated at 37 °C for 2 days. Bacterial growth was purified and then characterized based on elevation, edges, color, shape of bacterial colonies, and optical appearances, such as cell walls and shapes of bacterial cells [15]. Purified bacterial isolates were inoculated using the continuous streak plate method on NA medium, supplemented with Cu. The Cu concentrations used were 50, 100, and 150 mg/L. The culture were then incubated for 24 h at room temperature. Colony growth indicated that resistance to Cu metals was isolated. Isolates grow at a concentration of 150 mg/L were selected for further research. Isolation of bacterial DNA using the Quick-DNA Fungal/Bacteria Minirep kit. Bacterial DNA amplification was carried out using 16S rRNA primer, 27F and 1492R [16]. DNA sequencing was performed using Sanger Sequencing. The results of the bacterial 16S rRNA DNA sequencing were analyzed using the Basic Local Alignment Search Tool (BLAST) from NCBI (https://www.ncbi.nlm.nih.gov/). Phylogenetic tree construction was made using software called (MEGA) version 10.0 [17].

2.2 Test the ability of bacteria to remove copper metals

20 ml bacterial isolates were inoculated on to NB (80 ml incubated at 150 rpm for 24 h). A bacterial starter culture (100 ml was inoculated aseptically into 100 ml of nutrient broth liquid medium and incubated for up to 12 h. The starter culture on NB contained 50, 100, and 150) mg/L Cu up to a volume of 25 ml. Incubation was performed on a rotary shaker (150 rpm) for 24 h. The culture was centrifuged at 4500 rpm for 5 min to separate the cells from the filtrate that was collected and put into a test tube by; adding HNO_3. Cu metal was analyzed

using an atomic absorption spectrophotometer [18]. The percentage of heavy metals absorbed by the bacteria was calculated as follows [19]*:*

$$Q\,\% = \frac{Ci - Cf}{Ci}\ x\ 100\%$$

Notes:
Ci = initial concentration
Cf = final concentration of metal ions.

2.3 Scanning electron microscopy analysis

The surface morphology of the bacterial cells after the biosorption test for heavy metals was analyzed using scanning electron microscopy (SEM). Samples for observation using a scanning electron microscope were bacterial cells grown in nutrient broth media and bacterial cells grown in nutrient broth media containing copper metals. The bacterial isolates analyzed exhibited the highest ability to reduce copper.

3 Results and discussion

3.1 Morphology and identification of bacterial resistant to copper (Cu)

The isolation results from the Sayung Demak ditch showed two bacterial isolates that grew on Nutrient Agar media containing 150 mg/L of copper metal (Cu) YL18 and YL315. These bacterial colonies exhibited macroscopic and microscopic morphological differences. Microscopic observations showed that the isolates had an bacilli shape. Gram-negative bacteria were detected by Gram staining (Table 1). The Gram-negative bacteria in this study were able to survive in media with a concentration of 150 mg/L of Cu metal. Gram-negative bacteria contain lipopolysaccharides (LPS), proteins, and phospholipids. Lipopolysaccharides and phospholipids in the cell walls of gram-negative bacteria are composed of phosphate and carboxyl groups, respectively, which are compounds capable of binding metal ions [20].

Table 1. Morphological Characteristics of Bacteria

Isolate Code	Bacterial Colony Morphology						
	Colony Type					Microscopic	
	Color	**Margins**	**Elevation**	**Texture**	**Form**	**Grams**	**Cell Shape**
YL18	White	Lobalate	Flat	Moist	Irregular	Negative	Bacilli
YL315	Beige	Entire	Concex	Mucoid	Circular	Negative	Bacilli

The results of the YL18 and YL315 isolates were analyzed using the blast program on the NCBI (National Center of Biotechnology Information) website to align the DNA sequences of YL18 and YL315 isolates with the bacterial DNA sequence database available on the NCBI (National Center of Biotechnology Information) website. The results of this process provided information in the form of species that were closely related to isolates YL18 and YL315 (Table 2). Species derived from alignment with the NCBI database and bacterial isolates YL18 and YL315 were reconstructed into a phylogenetic tree, as shown in Fig 1.

Table 2. Blast Results

Isolate Code	Result Blast					
	Species Name	Max Score	Query Cover	E-Value	Identity	Accession
YL18	*Alcaligenes faecalis*	1779	100%	0	98,51%	NR_11360.1
YL315	*Delftia tsuruhatensis*	2562	100%	0	100%	MT374626.1

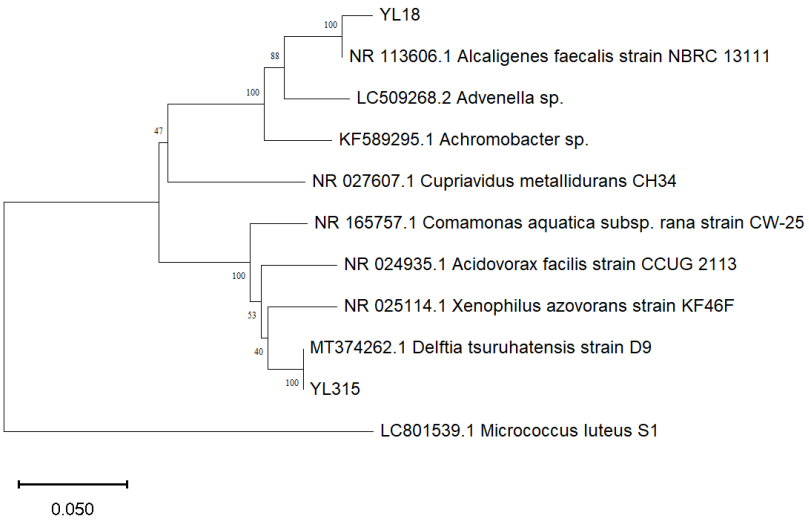

Fig 1. Phylogenetic tree reconstruction of bacterial isolates YL18 and YL315

The 16S rRNA gene isolate YL18 is the *species Alcaligenes faecalis*. The YL18 isolate derived from wastewater has characteristics of bacilli-shaped cells and gram-negative bacteria. The characteristics of *Alcaligenes faecalis* are rod-shaped (bacilli), gram-negative, aerobic, and polyvitric. Optimal growth of *Alcaligenes faecalis* bacteria isolated from waste water and soil at temperatures ranging from 20 to 37 °C [21]. The *Alcaligenes* genus can grow in environments containing Ni, Cd, Cu, Zn, Pb, and Cr. *Alcaligenes* contain bacterioferritin, porin, ABC transporters, and ATPase, which form a metal homeostasis system so that *Alcaligenes* survive in polluted environments [22]. The bacterial isolate, YL315, was identified as *Delftia tsuruhatensis*. *Delftia tsuruhatensis* is a rod-shaped gram-negative bacterium. *Delftia tsuruhatensis* was isolated from domestic wastewater in Japan [23]. *Delftia tsuruhatensis* has the genes copA, copB, copC, copF, copG, 2copR, copS, and cusA, which can live in an environment containing copper metal [24].

3.2 The ability of bacteria to remove copper

Biosorption testing of copper metals showed a decrease in metal concentrations in nutrient broth media without bacteria (Table 3). This decrease can be attributed to the presence of peptone in the nutrient broth medium, which is composed of amino acids containing reactive groups, such as carboxylic and amine groups [25]. The negatively charged carboxylate and amine groups can bind positively charged metals such as zinc and copper [26]. This causes the Nutrient Broth media to experience a decrease in the concentrations of zinc and copper metals. A decrease in metals in nutrient broth media without bacteria also occurred in a previous study [27] by as much as 24% at a concentration of 5 ppm.

Table 3. Concentration Media NB Without Bacteria

	Initial Concentration (mg/L)	**Final Concentration (mg/L)**
Copper (Cu)	50	1,024 (K1)
	100	1,027 (K2)
	150	1,454 (K3)

The test results for Cu metal removal showed that increasing the Cu metal concentration would decreased the absorption capacity (Table 4). The concentration of Cu without bacteria (50 mg/L) decreased to 1,024 mg/L, while in *Alcaligenes faecalis* bacteria it decreased to 1,1995 mg/L (Table 4). This condition shows that the final concentration of copper metal without bacteria is better than the addition of *Alcaligenes faecalis*, resulting in a negative copper metal reduction efficiency value (Table 4). The test results of *Alcaligenes fecalis* in absorbing copper metal show an upward and downward graph (Fig 2). The up and down values of metal absorption occurs because of the desorption ability of bacteria as a form of self-defense in environmental conditions that are not favorable for bacteria. Desorption is the process of releasing ions bound to the active groups on the adsorbent. In addition, it is also influenced by the surface area of the cell wall, aeration, and the specific gravity of the metal [28] .

Table 4. Ability of bacterial biosoption copper

Bacteria	**Initial Concentration (mg/L)**	**Final Concentration (mg/L)**	**Bioremoval Efficiency (%)**
Alcaligenes faecalis	1,024 (K1)	1,1995	-17%
	1,027 (K2)	0,625	39%
	1,454 (K3)	0,965	34%
Delftia tsuruhatensis	1,024 (K1)	0,349	66%
	1,027 (K2)	0,4635	55%
	1,454 (K3)	0,8015	45%

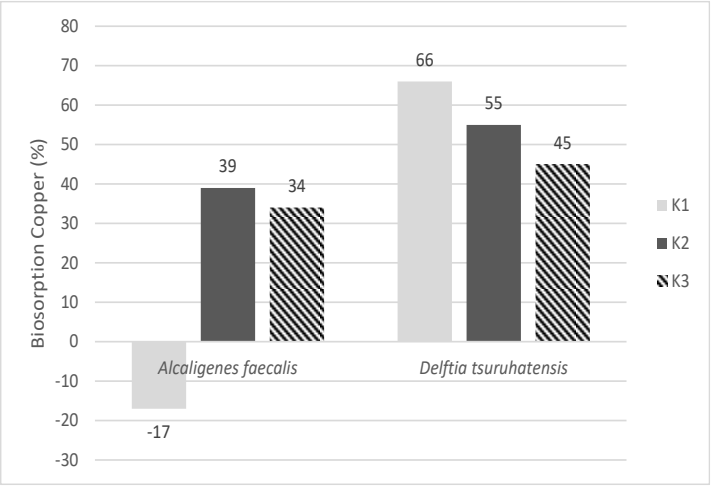

Fig 2. Bioremoval Efficiency of Copper by Bacterial Isolates

Delftia tsuruhatensis exhibited the highest copper reduction ability, with K3 showing the greatest reduction value of 45% (Fig 2). *Rhodococcus erythropolis* exhibited an absorption ability of 40% in copper media at a concentration of 0.5 mg/L. An increase in the concentration of copper metal causes a decrease in the percentage of copper removal [29]. The process of absorbing heavy metals by bacteria is carried out by ion exchange on cell surfaces, precipitation on cell and extracellular surfaces, physical adsorption on cell surfaces, complexation on cell surfaces, and transport of heavy metal ions [8].

3.3 Scanning electron microscopy analysis

The condition of *Delftia tsuruhatensis* cells before the addition of copper was observed using Scanning Electron Microscopy (Fig 3A and 3B). The results of the condition of *Delftia tsuruhatensis*, with copper metal (Cu), showed that the bacterial cells had an irregular cell shape, and there was a build-up of heavy metal molecules (Fig 3). The addition of metals to bacterial media can induce changes in the surface structure of the bacterial cells. Changes in the structure of bacterial cells in the presence of heavy metals were probably caused by the toxicity of metal ions on bacterial cell walls and the process of secretion of extracellular polymeric substances during metal biosorption [30]. In addition, a characteristic indicating the process of metal absorption is that the surface of the bacterial cells is covered with heavy metal molecules. This was possibly due to the process of secretion of extracellular polymeric substances or metabolism through metal binding to the cell surface, ion exchange, or precipitation [31].

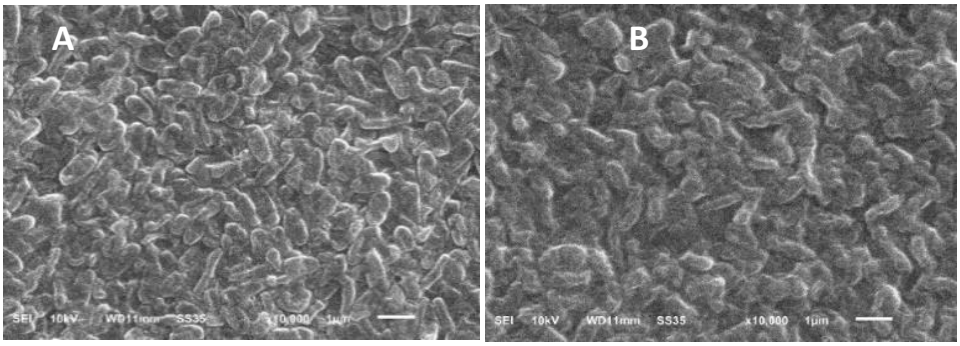

Fig 3. Cultured in liquid NB medium in the following treatments: A. control B. absence of metals Cu 50 mg/L

4 Conclusion

Alcaligenes faecalis and *Delftia tsuruhatensis* are indigenous Cu-resistant bacteria isolated from Sayung Demak. The ability of *Alcaligenes faecalis* to remove copper was 34% and *Delftia tsuruhatensis* was 45% at the highest concentration. The results showed that *Delftia tsuruhatensis* has the highest capacity for copper biosorption and condition bacterial cell added copper had an irregular cell shape, and there was a build-up of heavy metal molecules.

References

[1] C. N. Elizabeth, M. Y. Victoria, E. E. Nkechi, and B. O. Godwin, *African J. Biotechnol.*, **vol. 16**, no. 1, pp. 32–40, (2017), doi: 10.5897/ajb2016.15676.

[2] A. M. Elbedwehy, A. M. Abou-Elanwar, A. O. Ezzat, and A. M. Atta, *Polymers (Basel).*, **vol. 11**, no. 12, (2019), doi: 10.3390/polym11121938.

[3] S. Siddiquee, K. Rovina, and S. Al Azad, *J. Microb. Biochem. Technol.*, **vol. 07**, no. 06, pp. 384–393, (2015), doi: 10.4172/1948-5948.1000243.

[4] A. Abbas, S. Mohamed, and H. Zahir, *Int. Res. J. Eng. Technol.*, **vol. 03**, no. 08, pp. 1446–1450, (2016).

[5] O. B. Ojuederie and O. O. Babalola, *Int. J. Environ. Res. Public Health*, **vol. 14**, no. 12, (2017), doi: 10.3390/ijerph14121504.

[6] S. Srivastava, S. B. Agrawal, and M. K. Mondal, *Environ. Sci. Pollut. Res.*, **vol. 22**, no. 20, pp. 15386–15415, (2015), doi: 10.1007/s11356-015-5278-9.

[7] O. Abdi and M. Kazemi, *J. Mater. Environ. Sci.*, **vol. 6**, no. 5, pp. 1386–1399, (2015).

[8] E. A. Perpetuo and C. B. Souza, *Curr. Opin. Biotechnol.*, **vol. 11**, no. 3, pp. 262–270, (2011), doi: 10.1016/S0958-1669(00)00094-X.

[9] S. L. R. K. Kanamarlapudi, V. K. Chintalpudi, and S. Muddada, *Biosorption*, (2018), doi: 10.5772/intechopen.77315.

[10] M. Fomina and G. M. Gadd, *Bioresour. Technol.*, **vol. 160**, no. January 2014, pp. 3–14, (2014), doi: 10.1016/j.biortech.2013.12.102.

[11] H. Cerlyawati and S. Isworo, *Asian J. Biol.*, **vol. 13**, no. 115, pp. 34–51, (2021), doi: 10.9734/ajob/2021/v13i430194.

[12] L. Waluyo, *Pros. Semin. Nas. Pendidik. Biol.*, no. 2014, pp. 236–241, (2018).

[13] W. Irawati, R. Pinontoan, and T. Yuwono, *Biodiversitas*, **vol. 21**, no. 11, pp. 5077–5084, 2020, doi: 10.13057/biodiv/d211112.

[14] N. Z. S. Mazalan, A. Oyeleye, R. N. Z. R. A. Rahman, A. Z. Aris, A. B. Salleh, and Y. M. Normi, *Beni-Suef Univ. J. Basic Appl. Sci.*, **vol. 9**, no. 1, pp. 1–12, (2020), doi: 10.1186/s43088-020-00051-1.

[15] W. Irawati, *J. Biol. Papua*, **vol. 11**, no. 2, pp. 80–86, (2019), doi: 10.31957/jbp.878.

[16] G. Vasas *et al.*, *Toxins (Basel).*, **vol. 5**, no. 12, pp. 2434–2455, (2013), doi: 10.3390/toxins5122434.

[17] D. Ramya and A. J. Thatheyus, *Asian J. Biol. Sci.*, **vol. 12**, no. 4, pp. 869–876, 2019, doi: 10.3923/ajbs.(2019).869.876.

[18] A. Ahmad, R. Ghufran, and W. M. Faizal, *Clean - Soil, Air, Water*, **vol. 38**, no. 2, pp. 153–158, (2010), doi: 10.1002/clen.200900202.

[19] W. Mwandira *et al.*, *Sci. Rep.*, **vol. 10**, no. 1, pp. 1–9, (2020), doi: 10.1038/s41598-020-78187-4.

[20] S. Hussain *et al.*, *Front. Microbiol.*, **vol. 13**, no. May, (2022), doi: 10.3389/fmicb.2022.900740.

[21] É. B. Felestrino *et al.*, *PLoS One*, **vol. 15**, no. 11 November, pp. 1–25, (2020), doi: 10.1371/journal.pone.0241546.

[22] Z. Basharat, A. Yasmin, T. He, and Y. Tong, *Sci. Rep.*, **vol. 8**, no. 1, pp. 1–10, (2018), doi: 10.1038/s41598-018-21919-4.

[23] S. H. Al-Mijalli, *Appl. Environ. Soil Sci.*, **vol. 2022**, pp. 18–21, (2022), doi: 10.1155/2022/4316954.

[24] C. Cheng *et al.*, *Front. Cell. Infect. Microbiol.*, **vol. 11**, no. June, pp. 1–10, (2021), doi: 10.3389/fcimb.2021.663933.

[25] M. Vandenbossche, M. Jimenez, M. Casetta, and M. Traisnel, *Crit. Rev. Environ. Sci. Technol.*, **vol. 45**, no. 15, pp. 1644–1704, (2015), doi: 10.1080/10643389.2014.966425.

[26] V. Mishra, *Appl. Water Sci.*, **vol. 4**, no. 4, pp. 311–332, (2014), doi: 10.1007/s13201-013-0150-x.

[27] B. L. Fibriarti, N. P. Sari, and R. Fatzuarni, *Proceeding Biol. Educ. Conf.* , **vol. 15**, no. 1, pp. 880–882, (2018).

[28] F. S. Purnamawati, T. R. Soeprobowati, and M. Izzati, *Bioma*, (vol. 16), no. 2, pp. 102–113, (2015).

[29] M. D. P. G. Baltazar *et al.*, *J. Mater. Res. Technol.*, **vol. 8**, no. 1, pp. 475–483, (2019), doi: 10.1016/j.jmrt.2018.04.006.

[30] G. Pagnucco *et al.*, *Front. Microbiol.*, **vol. 14**, no. October, pp. 1–21, (2023), doi: 10.3389/fmicb.2023.1278886.

[31] S. Kumari *et al.*, *Separations*, **vol. 10**, no. 7, (2023), doi: 10.3390/separations10070393.