Volume 2, No. 7 July 2023 - (xx-xx)
p-ISSN xxxx-xxxx | e-ISSN 2980-4841
https://ajesh.ph/index.php/gp

MICROBIAL MERCURY REDUCTION POTENTIAL IN GOLD MINING WASTE: *BACILLUS SUBTILIS* AND *PSEUDOMONAS AERUGINOSA* STUDY

Sekarsari Wibowo [1)], **Ipung Fitri Purwanti** [2)]
Faculty Of Civil, Planning and Geo Engineering Sepuluh Nopember Institute of Technology, Indonesia
Emails: sekarsari.wibowo@gmail.com [1)]
fit_purwanti@yahoo.com [2)]

ABSTRACT:

This study aims to investigate the potential of *Bacillus subtilis* and *Pseudomonas aeruginosa* in removing mercury from wastewater generated by Artisanal and Small-scale Gold Mining (ASGM) in Tatelu and Talawaan Villages, North Minahasa, Indonesia. Following the ban on mercury use in gold processing, there is a need for effective mercury-free processing technologies to mitigate environmental and health impacts.

The research begins with the cultivation of rejuvenated bacteria, followed by a preliminary test to establish bacterial growth curves. This test provides guidelines for the duration of stirring during subsequent biodegradation processes. The biodegradation test involves mercury content analysis of wastewater samples, followed by Minimum Inhibitory Concentration (MIC) testing using lactose broth as the bacterial growth medium. The MIC test helps determine the variations in mercury concentrations and the efficacy of the selected bacteria in degradation.

The objectives of this study are to assess the capability of *Bacillus subtilis* and *Pseudomonas aeruginosa* in removing mercury from ASGM wastewater. The findings hold significant potential for the development of efficient and economically viable biotreatment technologies for addressing mercury contamination in hazardous waste produced by small-scale gold mining. The innovative technology proposed in this study can be readily adopted by other gold mining industries to ensure compliance with stringent environmental and health standards.

Keywords: **Artisanal and Small-scale Gold Mining, *Bacillus subtilis*, biodegradation, mercury removal, minimum inhibitory concentration test, *Pseudomonas aeruginosa*, wastewater treatment**

Article History
Received : 6 July 2023
Revised : 22 July 2023
Accepted : 25 July 2023

DOI : 10.46799/ajesh.v2i7.77

INTRODUCTION

T In 2017, a ban on the use of mercury in gold processing was implemented, based on Undang-undang No. 11/2017 concerning the Ratification of the Minamata Convention on Mercury or Law No. 11/2017, along with its derivative regulation, Presidential Regulation Number 21 of 2019 concerning the National Action Plan for the Reduction and Elimination of Mercury, abbreviated as RAN PPM. The goal of this plan is to discover mercury-free gold processing technologies accompanied by environmental impact management, as well as the application of appropriate and effective mercury-free gold processing technologies that can improve gold recovery outcomes (Pratiwi, 2020).

The idea for studying biotreatment of wastewater resulting from gold mining activities was derived from the PESK locations in the villages of Tatelo and Talawaan, North Minahasa Regency, North Sulawesi Province. The selection of these locations was based on several considerations, including the presence of Small-Scale Mining Permits (IPR) in the Artisanal Mining Area (WPR) and the ongoing gold mining activities in the area.

Numerous studies have conducted physical and chemical testing on various methods of treating wastewater containing inorganic heavy metals (Bots, M. *et al*., 2016). However, some of these treatment methods are considered ineffective when compared to biotreatment, which can effectively remove heavy metal compounds within a wide concentration range (Dash, R.R. *et al*., 2009). According to Gurbuz, F. *et al*. (2009), biotreatment, or biological treatment methods, are economical and effective in reducing the toxicity and corrosive nature of oxidative chemicals. Therefore, the development of biotreatment for wastewater contaminated with heavy metals is crucial in the SSGM industry.

In addition, bioremediation methods can also be integrated with other techniques such as physicochemical treatment and membrane separation to enhance the mercury reduction process in wastewater from SSGM. Therefore, the use of bioremediation methods utilizing *Bacillus subtilis* and *Pseudomonas aeruginosa* bacteria has the potential to provide an effective and environmentally friendly solution for managing SSGM wastewater in the future.

RESEARCH METHODS

Preparation of media solutions

All chemicals and media used were analytical grade reagents. Liquid and agar media were respectively made using nutrient broth (NB) and nutrient agar (NA) (Merck, Germany). The liquid medium was prepared from 13 g of NB powder in 1 L distilled water. Mean- while, the agar medium was made with 20 g of NA powder in 1 L

distilled water and then heated until dissolved.

Preparation of stock solutions

In this experiment, artificial samples, the concentrations of mercury used in this study were 0.25, 0.5, 0.75, 1, 1.5, 100, and 1000 ppm. These concentrations were chosen based on the mercury concentrations found in the wastewater from small-scale gold mining activities using the cyanidation method in the villages of Tatelu and Talawaan, North Minahasa Regency and represents the typical mercury concentrations found in the wastewater of small-scale gold mining activities in Indonesia that utilize the gold amalgamation method with mercury as the main component.

0.1354 grams of HgCl2 was weighed and transferred to a measuring glass, followed by the addition of 1 ml of 65% HNO3. The mixture was then diluted with distilled water (aquades) up to the 50 ml mark. The solution was transferred to a 100 ml volumetric flask and further diluted with 50 ml of aquades. Thus, a mercury (Hg) solution with a concentration of 1000 ppm was obtained. This solution can be further diluted with aquades to achieve the desired concentrations of mercury needed for the minimum inhibitory concentration experiment.

Bacterial Rejuvenation

During this stage, the bacteria that being used in this study are rejuvenated to ensure the viability and prevent contamination of the bacterial culture. The rejuvenation process involves aseptically inoculating the bacteria using an inoculating loop onto fresh Nutrient Agar (NA) media within a laminar airflow hood. The inoculated plates are then incubated at 37°C in a bacteriological incubator for 24 hours. The rejuvenation process provides a reserve of bacterial cultures in case of errors or unexpected needs during the study. This process is carried out to prepare the bacteria prior to their use in the research procedures.

Minimum Inhibitory Concentration (MIC) assessment

The Minimum Inhibitory Concentrations (MIC) test was conducted using tailing samples derived from gold mining activities as the medium for pollutant exposure. The MIC evaluation for each bacterial isolate in this research employed the widely recognized disk diffusion method, as described by Pundogar *et al*. (2014). To initiate the MIC testing, each bacterial isolate was meticulously reintroduced into 250 ml Erlenmeyer flasks containing 100 ml of nutrient broth, ensuring strict aseptic conditions. The flasks were subsequently placed within an incubator shaker, operating at a controlled temperature of 37°C and a shaking speed of 150 rpm. The incubation period was precisely determined based on the results derived from the logarithmic growth curve of the respective bacterial isolates. In this study, the optimal incubation duration was determined to be three hours. Subsequently, 0.1 ml of each bacterial inoculum was meticulously transferred onto sterilized disks pre-coated with nutrient agar, enabling the subsequent MIC assessment.

The investigation involved the evaluation of the inhibitory effects of mercury on the bacterial isolates using seven different concentrations: 0.25, 0.5, 0.75, 1,

1.5, 100, and 1000 ppm. These concentrations were carefully chosen to encompass the typical range of mercury levels found in wastewater derived from gold processing activities, including both environmentally conscious cyanidation methods and conventional gold amalgamation techniques employing mercury. By incorporating a diverse set of concentrations, the study aimed to comprehensively examine the responses of the bacterial isolates to varying levels of mercury-induced toxicity. The objective was to establish the minimum inhibitory concentrations (MICs) for each isolate, providing valuable insights into their sensitivity and potential for application in bioremediation efforts within mercury-contaminated environments.

To assess the minimum inhibitory concentrations (MICs) of the bacterial isolates towards mercury, sterilized filter paper discs were immersed in 20 ml of each mercury concentration for a duration of one hour. Following the immersion period, the discs were carefully placed onto the surface of nutrient agar plates. Subsequently, the plates were incubated at 37°C for 24 hours to allow for the development of visible inhibition zones. The determination of MIC values for each bacterial isolate was based on the lowest concentration of mercury that resulted in the formation of an inhibitory zone on the nutrient agar surface. The diameter of the clear zone indicated the extent to which microbial activity was hindered by the presence of mercury at a specific concentration. Thus, the minimum inhibitory concentration could be determined by measuring the diameter of the inhibition zone (Jayanthi *et al*., 2016).

This experimental procedure allowed for a systematic evaluation of the bacterial isolates' susceptibility to mercury toxicity. By immersing the filter paper discs in various concentrations of mercury and subsequently measuring the diameter of the inhibition zones, the study aimed to identify the precise concentration at which mercury significantly impacted the growth and survival of the tested bacteria. This approach enabled the determination of MIC values for each bacterial isolate, providing valuable insights into their tolerance levels and aiding in the assessment of their potential for bioremediation applications in mercury-contaminated environments.

RESULTS AND DISCUSSION

Development of Bacterial Growth Curves

Monitoring bacterial growth in liquid culture is done by measuring the optical density at 600 nm, called OD600. This helps track growth stages and ensure optimal cell density during harvest. Bacterial growth occurs in lag, log, stationary, and decline phases. OD600 indicates the current phase and growth dynamics. It minimizes cellular damage and provides accurate results without interfering with cell viability. Harvesting cells at the end of the logarithmic phase, determined by OD600, ensures high density and physiological state for downstream applications. OD600 enables rigorous and accessible monitoring of bacterial growth behavior and dynamics.

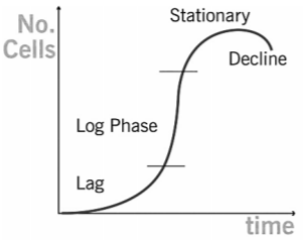

Figure 1. Bacterial Growth Curve

Bacterial growth involves lag, log, stationary, and decline phases. The log phase is critical for active cell division and maximum growth rate. Monitoring optical density at 600 nm (OD600) helps assess cell density and optimal growth point. Optical density quantifies light scattering by cells, indicating higher density with higher optical density values. Tracking optical density allows researchers to analyze growth dynamics and determine the ideal time for cell harvest. Harvesting during the late logarithmic phase ensures high density and optimal physiological state for downstream applications. Optical density measurements, specifically OD600, provide a non-invasive and convenient method to monitor growth, assess population density, and optimize experimental outcomes.

Prior to conducting the bioaugmentation process, a bacterial growth curve was generated to determine the growth rate of each bacterium. This information is crucial for designing an effective bioaugmentation strategy for the management of toxic mercury-containing waste.

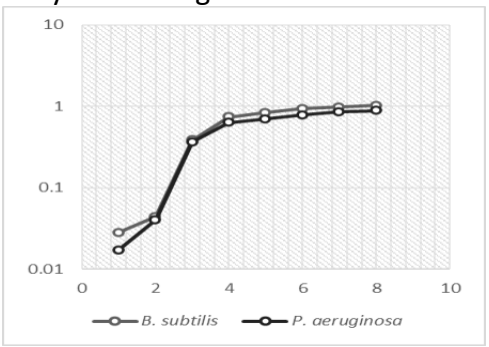

Figure 2. Bacterial Growth Curve

Source: Primary data, 2023

Both *Bacillus subtilis* and *Pseudomonas aeruginosa* exhibit exponential growth starting from the 2nd hour, characterized by a rapid increase in absorbance values. During this phase, cells divide rapidly and secrete enzymes for energy. Exponential phase is characterized by rapid cell division (Kane and Kandel, 1998). *Bacillus subtilis* enters stationary phase at 9th hour, while *Pseudomonas aeruginosa* continues exponential growth until the 19th hour (Arfiati *et al*., 2020; Damayanti, 2016). Lag phase duration depends on factors like initial inoculum size and nutrient composition (Dahlan *et al*., 2017). Based on the growth curves, a 3-hour shaking process will be conducted before MIC test, aligning with the midpoint of exponential phase (Arfiati *et al*., 2020; Damayanti, 2016).

Minimum Inhibitory Concentration (MIC) Assessment

The MIC test in this study employed the disk diffusion method to determine the lowest concentration of mercury that inhibited bacterial growth on nutrient agar (Pundogar *et al*., 2014; Jayanthi *et al*., 2016). The size of the inhibitory zone increased with higher mercury concentrations, indicating reduced microbial activity (Imron *et al*., 2019). Bacteria can be categorized as sensitive, intermediate, or resistant to mercury based on their MIC values. Lower MIC values indicate higher sensitivity, while higher values suggest resistance. Intermediate category indicates moderate to low sensitivity to mercury (Imron *et al*., 2019).

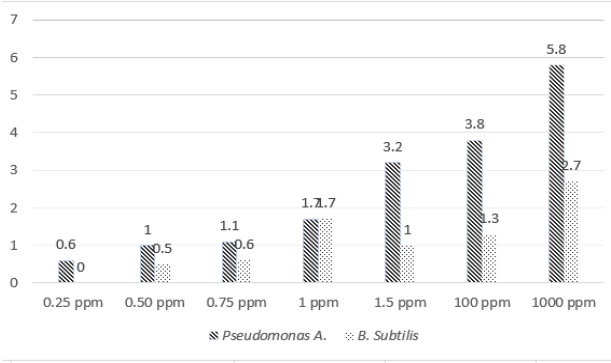

Figure 3. The Final Results of The MIC Test

Source: Primary data, 2023

In this study, *Bacillus subtilis* and *Pseudomonas aeruginosa* exhibited successful growth in the presence of a mercury concentration of 0.25 ppm, as indicated by the formation of clear zones around the filter paper discs, signifying inhibited microbial growth (Imron *et al*., 2019). This concentration was determined to be optimal for the growth of both bacteria. The results support the notion that increasing exposure to mercury pollutants can diminish bacterial survival and growth potential. The ability of these bacteria to grow and absorb mercury further emphasizes their potential as safe biotreatment agents for environments contaminated with heavy metal pollutants.

In conclusion, the MIC test revealed that mercury concentrations significantly influenced bacterial growth, with lower MIC values indicating greater sensitivity and higher values indicating resistance. *Bacillus subtilis* and *Pseudomonas aeruginosa* exhibited resistance to mercury at concentrations other than 0.25 ppm, while showing successful growth and mercury absorption at this optimal concentration. These findings highlight the potential of these bacteria for biotreatment applications in mercury-contaminated environments. Increased exposure to mercury can pose a threat to bacterial survival and growth, emphasizing the importance of understanding the effects of heavy metal pollutants on microbial ecosystems.

CONCLUSION

In conclusion, *Bacillus subtilis* and *Pseudomonas aeruginosa* demonstrated resistance to mercury pollutants and showed the ability to absorb mercury, particularly at a concentration of 0.25 ppm. These bacterial strains hold promise as safe and effective biotreatment agents for mercury-contaminated environments, specifically in the context of artisanal and small-scale gold mining (ASGM) wastewater treatment. Future research should focus on optimizing conditions for mercury removal, understanding the mechanisms behind their resistance, and exploring the scalability and cost-effectiveness of implementing these bacteria in ASGM wastewater treatment facilities. This study contributes to the development of sustainable approaches for mitigating mercury pollution and reducing the negative impacts of ASGM on ecosystems and human health.

BIBLIOGRAFI

1. Arfiati, D., Shofiyatul, L., Karina, F.D., Nunik, C. (2020). Dinamika Jumlah Bakteri Bacillus Subtilis dalam Penurunan Kadar Bahan Organik TOM Limbah Budidaya Ikan Lele Sangkuriang (Clarias gariepinus). Journal of Fisheries and Marine Research Vol. 4 No. 2 (2020): 222-226
2. Dahlan, A., Wahyuni, S., Ansharullah. (2017). Morfologi dan Karakterisasi Pertumbuhan Bakteri Asam Laktat (UM 1,3A) dari Proses Fermentasi Wikau

Maombo untuk Studi Awal Produksi Enzim Amilase. Jurnal Sains dan Teknologi Pangan, 2(4):657-663

3. Damayanti, N.F. (2016). Pengaruh Penambahan Bakteri Pseudomonas aeruginosa terhadap Biodegradasi DDT oleh Jamur Pelapuk Coklat Gleophyllum trabeum. Skripsi. Jurusan Kimia Fakultass Matematika dan IPA Institut Teknologi Sepuluh Nopember
4. Gurbuz, F., Ciftci, H. and Akcil, A. 2009. Biodegradation of Cyanide Containing Effluents by Scenedesmus obliquus. Journal of Hazardous Materials, 162, 74-79.
5. Imron MF, Kurniawan SB, Soegianto A. Characterization of mercury-reducing potential bacteria isolated from Keputih non-active sanitary landfill leachate, Surabaya, Indonesia under different saline conditions. J Environ Manage. 2019;241:113–22.
6. Jayanthi, B., Emenike, C.U., Auta, S.H., Agamuthu, P., Fauziah, S.H., 2016b. Characterization of induced metal responses of bacteria isolates from active non-sanitary landfill in Malaysia. Int. Biodeterior. Biodegrad. 119, 467–475. https://doi.org/10.1016/j.ibiod.2016.10.053
7. Pratiwi D. Y., “Dampak pencemaran logam berat (timbal, tembaga, merkuri, kadmium, krom) terhadap organisme perairan dan Kesehatan manusia,” J. Akuatek, vol. 1, no. 1, pp. 59–65, 2020