JOURNAL OF WATER AND LAND DEVELOPMENT
e-ISSN 2083-4535
Polish Academy of Sciences (PAN) Institute of Technology and Life Sciences – National Research Institute (ITP – PIB)

JOURNAL OF WATER AND LAND DEVELOPMENT
DOI: 10.24425/jwld.2025.154264
2025, No. 65 (IV–VI): 201–208

Bioleaching potential of local bacteria from gold mine tailings in the Ratatotok Area, Indonesia

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RECEIVED 29.12.2024 ACCEPTED 27.03.2025 AVAILABLE ONLINE 13.06.2025

Abstract: Our previous study found that *Bacillus cereus* from the rhizosphere of *Syzygium aromaticum* (RTKS) exhibited the highest resistance and the best gold bioleaching potential at pH 8. However, this bacteria's potential requires further investigation. The current study aimed to (1) determine the temperature that supports the effectiveness of *B. cereus* RTKS in the bioleaching of gold, and (2) test the effectiveness of bioleaching by *B. cereus* RTKS with variations in Au contents in tailings. The experiment began by growing bacteria in a medium, which was then inoculated into tailing effluent. The experiment was carried out in an Erlenmeyer flask shaken at 120 rpm for 3 weeks. In the first-stage experiment, temperatures of 25°C and 50°C were tested. The optimum temperature obtained in the first stage was then used for the second-stage experiment with variations in Au contents in tailings. After bioleaching, the filtration process was conducted and produced three components, namely pellets containing bacterial cells, supernatants, and residues. Bacteria mentioned above were more effective in bioleaching of gold at a temperature of 50°C than at 25°C, indicated by higher Au extraction in pellets and residues, and Au accumulation which tended to be higher in pellets. In treatments with varying Au concentrations in the tailings, increasing Au content did not lead to higher Au accumulation by *B. cereus* RTKS. The Au content in the residue was significantly highest in the tailings bioleaching treatment with the highest Au content.

Keywords: *Bacillus cereus* RTKS, bioleaching ability, bioleaching effectiveness, gold, tailing

INTRODUCTION

Ratatotok District, Southeast Minahasa Regency, North Sulawesi Province, Indonesia, is an area that was once gold-producing and still contains relatively high gold concentrations, ranging from 0.012 g·Mg^{-1} to 2.41 g·Mg^{-1} (Azzaman, Idrus and Titisari, 2021). In 2022, we conducted a study at the Ratatotok mining site to obtain information on the type of gold deposits, the concentration of gold in the soil, types of vegetation that can thrive at the site (have a high importance value index) at the mining site location, and then to isolate rhizobacteria (bacteria that live in the rhizosphere of dominant vegetation).

The results of the previous study revealed the gold deposits in Ratatotok are of the refractory type, stored in sulphide ore, making gold particles invisible ("invisible gold"). We found three types of plants with high importance value indices and moderate bioaccumulation capabilities, as indicated by bioconcentration factors (*BCF*) ranging from over 0.1 to 1.0. These species are *Pteris vitata*, *Syzygium aromaticum*, and *Swietenia mahagoni*. In contrast, the other plant species have low bioaccumulation capabilities ($BCF < 0.1$) (Aminatun *et al.*, 2024b). A *BCF* value $\in (0.1; 1.0)$ falls within the moderate category (Yoon *et al.*, 2006; Herlina *et al.*, 2020; Kurniawan *et al.*, 2022). The ability of these plants to tolerate metal stress may be attributed to the presence of rhizosphere bacteria that live in plant roots. They produce secondary metabolites such as indole acetic acid (IAA) or other growth triggers and siderophore compounds, which can chelate metals in the soil and transport them into plant cells (Govarthanan *et al.*, 2016). In 2022, we successfully isolated bacterial colonies from the rhizosphere of the three dominant plant species. We recorded $39 \cdot 10^{15}$ cfu·g^{-1} from *Pteris vittata*, $44.8 \cdot 10^{17}$ cfu·g^{-1} from *Syzygium aromaticum* (L.) Merr. & L.M.Perry, and $61 \cdot 10^{14}$ cfu·g^{-1} from *Swietenia mahagoni* (L.) Jacq. It is suspected that these bacterial colonies affect the phytoextraction and bioaccumulation of gold in these vegetation biomasses, so they have the potential as bioleaching agents (Aminatun *et al.*, 2024a).

Invisible gold refers to gold that is difficult to extract, typically requiring the use of cyanide acid. However, cyanide is a toxic compound that can harm the environment and can harm the environmental and lead to ecological disasters (Gani, Abidjulu and Wuntu, 2017; Muyassaroh and Salami, 2020). Bioleaching, the extraction of gold with the help of bacteria, offers an environmentally friendly alternative. However, inducing exogenous or non-native bacteria into the bioleaching process can lead to competition with native bacteria, potentially causing negative effects (Phyo *et al.*, 2020). Therefore, the use of local bacteria is preferable. Thus, it is essential to explore the use of local bacteria, especially rhizobacteria, associated with accumulator plants in metal-stressed mining areas.

We characterised the phenotypic traits of rhizobacteria isolates obtained in the previous 2022 study. The results showed that all isolates produce IAA hormone. Based on genotypic characterisation, the three isolates most resistant to tailings stress are *Pseudomonas aeruginosa* RTKP1 and *Stenotrophomonas geniculata* RTKP2 (both from the rhizosphere of *Pteris vittata*), and *Bacillus cereus* RTKS (from the rhizosphere of *Syzygium aromaticum*). Among them, *Bacillus cereus* RTKS exhibited the highest resistance and the most effective leaching ability in alkaline media (pH = 8) (Aminatun *et al.*, 2024a). However, the bioleaching potential of *Bacillus cereus* RTKS requires further investigation, especially concerning its ability in various environmental conditions and at various tailing concentrations. Therefore, the current study aimed to (1) determine environmental conditions that support the effectiveness of *Bacillus cereus* RTKS in bioleaching, and (2) evaluate its bioleaching efficiency at various tailing concentrations.

Although bioleaching research using *Bacillus cereus* has been conducted previously (Yin *et al.*, 2019), that study used local strains from mining areas in Mexico and focused only on bioleaching of Arsenic (As). Another study investigated gold bioleaching with local bacteria, but it employed *Alcaligenes faecalis* isolated from mining sites in California (Cabrales-González *et al.*, 2022). Neither study examined bacterial isolates originating from the rhizosphere of dominant vegetation in gold mining areas. Therefore, the current study presents several novelties, including the source of bacterial isolates, bacterial strains, and elements treated, as well as the use of different research methods.

MATERIALS AND METHODS

This study involved field exploration and advanced bioleaching laboratory. Field exploration was conducted at the Ratatotok gold mine site, Southeast Minahasa, North Sulawesi, Indonesia, and included observation and sampling of tailings (spent ore). Geochemical analysis and characterisation of tailings were conducted in an accredited laboratory of the PT Indo Mineral Research, Purwakarta Regency, West Java, Indonesia. These analyses included Au tests using FA-AAS and Multielements ICPMS Package tests. Bioleaching experiments were conducted in the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Negeri Yogyakarta, Indonesia.

Furthermore, in more detail the following research stages.

1. Sampling of tailings (spent ore).

Gold mine tailings (spent ore) samples were collected from the spent ore stockpile at the official Ratatotok mining site, Southeast Minahasa Regency, North Sulawesi, Indonesia (Fig. 1) for later preparation.

2. Sample preparation and geochemical testing.

Representative samples were dried in an oven at 110°C for 6–7 h, then crushed using a primary jaw crusher and roll crushing. Then, quartering (homogenisation) and refining (pulverising) were carried out until the samples reached a fine grain size of 200 mesh (pulp). Preparation of tailing samples (spent ore) was carried out in a laboratory owned by PT Sumber Energi Jaya (PT SEJ) at the Ratatotok site. The resulting pulp was then used for bioleaching experiments and tailing geochemical characterisation, including Au tests with FA-AAS and Multielements ICP-MS Package tests (in an accredited laboratory of the PT Indo Mineral Research, Purwakarta Regency, West Java, Indonesia). The multielement analysis included Cu, Ag, Sb, and As, as these elements are closely associated with the Au mineralisation process, especially in Carlin-type or refractory deposits (Kampmann *et al.*, 2018; Kudrin *et al.*, 2021; Zhang *et al.*, 2022).

3. Test of physical environmental conditions (temperature) that support the effectiveness of *Bacillus cereus* RTKS bacteria for gold bioleaching.

Bacillus cereus RTKS, isolated and characterised in the previous study in 2023, was used in an augmented bioleaching experiment. The experiment began by growing bacteria in a medium, which was then inoculated into tailings waste. The experiment was carried out in an Erlenmeyer flasks, shaken at 120 rpm for 3 weeks (21 days). The experiment tested two temperature conditions: room temperature (25°C) and 50°C. Temperature was selected as a key parameter due to its significant influence on the adsorption, leaching, and oxidation of metal-binding sulphides (Villares *et al.*, 2016).

4. Test of bioleaching effectiveness by *Bacillus cereus* RTKS at various Au contents in tailings.

After obtaining the optimum temperature condition for the bioleaching process in stage 3, the optimum temperature condition was used for bioleaching experiments involving

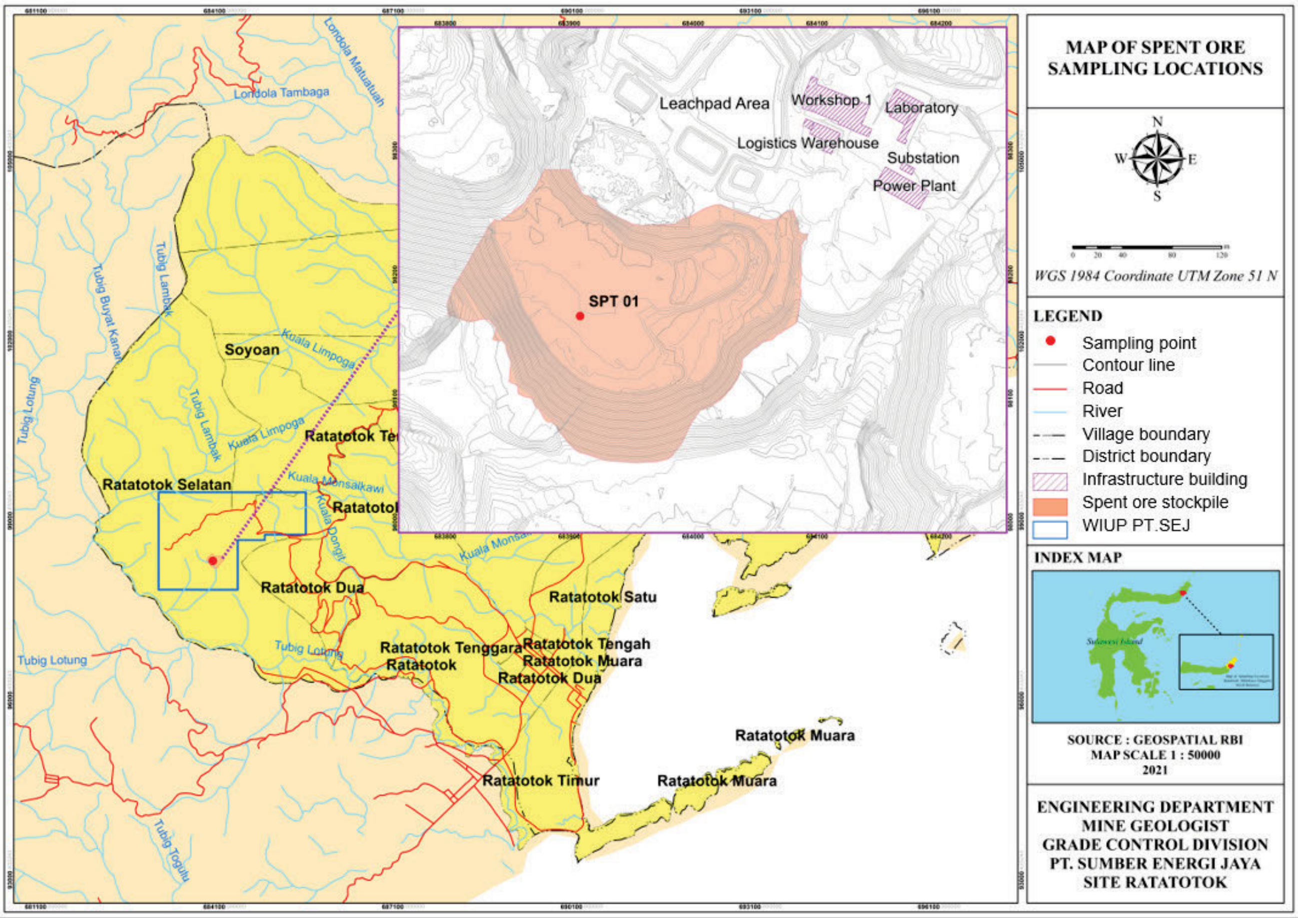

Fig. 1. Map of tailing sampling at the Ratatotok gold mining site, North Sulawesi, Indonesia; source: own elaboration

variations in tailing concentrations. The bioleaching experimental procedure was the same as in the previous stage, with the only difference being the variation in tailing concentrations, based on differing initial gold content levels. The bioleaching performance was assessed by measuring the concentration of gold successfully leached/released from sulphide mineral bonds at each tailing concentration.

5. Data analysis.

Quantitative data analysis was conducted descriptively by comparing the mass balance of Au and multielements between treatments before and after bioleaching treatments. The mass balance calculation was carried out using the Equations (1)–(6).

Calculating the head or mass balance of Au and multi-elements (*Hd*) before bioleaching:

$$Hd = \frac{AM_0 \cdot TM}{1000} \tag{1}$$

where: AM_0 = initial content of Au or multielements (mg·kg^{-1}), *TM* = tailing mass (g).

Calculating mass balance of Au and multielements in pellet components (*AP*) after bioleaching:

$$AP = \frac{AM_{P1} \cdot PM}{1000} \tag{2}$$

where: AM_{P1} = content of Au or multielements in pellet (mg·kg^{-1}), *PM* = pellet mass (g).

Calculating mass balance of Au and multielements in supernatant components (*AS*) after bioleaching:

$$AS = \frac{AM_{S1} \cdot SM}{1000} \tag{3}$$

where: AM_{S1} = content of Au or multielements in supernatant (mg·kg^{-1}), *SM* = supernatant volume (cm^3).

Calculating mass balance of Au and multielements in residue components (*AR*) after bioleaching:

$$AR = \frac{AM_{R1} \cdot RM}{1000} \tag{4}$$

where: AM_{R1} = content of Au or multielements in residue (mg·kg^{-1}), *RM* = residue mass (g).

Calculating back-extraction (*BE*, %):

$$BE = \frac{AM_{P1} \text{ or } AM_{S1} \text{ or } AM_{R1}}{(AM_{P1} + AM_{S1} + AM_{R1})} 100 \tag{5}$$

Calculating head extraction (*HdE*, %):

$$HdE = \frac{AM_{P1} \text{ or } AM_{S1} \text{ or } AM_{R1}}{Hd} 100 \tag{6}$$

The difference test of Au mass balance between treatments was carried out using ANOVA.

RESULTS

GEOCHEMICAL CHARACTERISTICS OF TAILINGS BEFORE BIOLEACHING

Before the Au bioleaching test was carried out with *Bacillus cereus* RTKS, the tailing samples were tested for their Au and multielement contents. The results of which are presented in Table 1.

Table 1. Gold and multielement content before bioleaching

No.	Element	Content (ppm)
1	Au	0.580
2	Ag	1.905
3	Cu	22.462
4	Sb	220.554
5	As	1,100

Source: own study.

Based on Table 1, the tailing sample contained 0.580 ppm of Au. This tailing sample was then used in treatment tests under different temperatures, namely room temperature (25°C) and 50°C. Tailing samples also contained elements such as Ag, Cu, Sb, and As, often associated in nature with gold in this type of deposit. Field observations and microscopic tests showed that Cu originated from chalcopyrite, Sb – from stibnite, and high levels of As (1,100 ppm) – from pyrite containing high arsenic called arsenian pyrite (Hofstra and Cline, 2020).

RESULTS OF THE FIRST-STAGE BIOLEACHING TREATMENT WITH VARIATIONS IN TEMPERATURE

The most effective temperature condition in the first-stage bioleaching treatment was defined as the one resulted in the highest percentage of Au released from the tailings and the greatest bioaccumulation of Au in bacterial cells. The bioleaching treatment produced three components, namely tailing residue, pellet containing bacterial cells, and supernatant. The separation of the three components was done through a 2-fold filtering process, first using 400-mesh filter paper, followed by 600-mesh filter paper. Furthermore, the three components were analysed to determine the Au and multielement contents. The results are presented in Tables 2–4.

Based on the average mass balance calculation, the highest Au mass in the pellet component was found at the 50°C treatment, amounting to 0.00049 mg. The highest Au mass in the residue component was recorded at 50°C, which was 0.0209 mg. In contrast, the highest Au mass in the supernatant component was found at 25°C, reaching 0.00193 mg. These results were then used to calculate the percentage of Au extraction, as shown in Table 3.

Based on the extraction calculations, the optimal treatment was at 50ᵒC, as it resulted in the highest percentage of Au extraction in the pellet component. In addition, the loss rate at this temperature was the lowest. Table 4 presents the average mass balance of multielements following the first-stage bioleaching experiment. Based on the first-stage bioleaching experiment with variations of temperature, the optimum temperature with the best results was 50°C, based on both Au content and Au extraction efficiency in the pellet component. Furthermore, the optimum temperature (50°C) was used in the second-stage bioleaching experiment, which tested different tailing samples with varying Au contents: 0.58 ppm (code PSO1), 0.63 ppm (code PSO2), and 0.97 ppm (code PSO3).

Table 2. Average mass balance of gold after the first-stage bioleaching experiment

Component	Au mass (mg·kg^{-1}) temperature treatment	
	25°C	50°C
Pellet	0.000266667	0.000486667
Residue	0.01818	0.020923333
Supernatant	0.00193	0.00131

Source: own study.

Table 3. Average gold extraction after the first-stage bioleaching experiment

Temperature treatment	Average Au tailing extraction in components (%)			Loss (%)
	pellet	residue	supernatant	
25°C	0.45	31.11	3.30	65.13
50°C	0.83	35.82	2.24	61.11

Source: own study.

Table 4. Average mass balance of multielements in the residue after the first-stage bioleaching experiment

Element	Average mass balance (mg) after bioleaching at	
	25°C	50°C
Ag	0.082	0.091
As	70.567	83.026
Cu	0.962	1.050
Sb	11.970	14.594

Source: own study.

RESULTS OF THE SECOND-STAGE BIOLEACHING TREATMENT WITH VARIATIONS IN TAILING GOLD CONTENTS

The most effective treatment was defined as the one that resulted in the highest percentage of Au released from the tailings and the greatest bioaccumulation of Au in bacterial cells. The second-stage bioleaching experiment also produced three components: tailing residue, pellet containing bacterial cells, and supernatant. Furthermore, each of the three components was tested for Au and multielement content. The results are shown in Table 5.

The results in Table 5 were then used to calculate the percentages of Au extraction, which are shown in Table 6.

Table 5. Average mass balance of gold after the second-stage bioleaching experiment

Tailing code	Average mass balance of Au after the second-stage bioleaching experiment (mg)		
	pellet	residue	supernatant
PSO1	0.0007	0.0206	0.0064
PSO2	0.0010	0.0212	0.0013
PSO3	0.0014	0.0365	0.0025

Explanations: PSO1 = Au content of 0.58 ppm, PSO2 = Au content of 0.63 ppm, and PSO3 = Au content of 0.97 ppm.
Source: own study.

Table 6. Average gold extraction after the second-stage bioleaching experiment

Tailing code	Average Au tailings extraction in components (%)			Loss (%)
	pellet	residue	supernatant	
PSO1	1.23	35.33	10.90	52.55
PSO2	1.59	33.71	2.06	62.65
PSO3	1.46	40.11	2.62	55.81

Explanations as in Tab. 5.
Source: own study.

Based on the extraction calculations, the PSO2 treatment yielded the highest percentage of Au extraction in the pellet component. PSO3 showed the highest percentage of Au extraction in the supernatant component, while PSO1 had the highest Au extraction in the residue component. However, when considering the lowest loss rate, PSO1 was identified as the most effective treatment. The results of the Au content analysis following the second-stage bioleaching experiment are presented in Table 7.

Table 7. Average gold content after the second-stage bioleaching experiment

Tailing code	Average Au content (ppm)		
	pellet	residue	supernatant
PSO1	1.21	0.26	0.06
PSO2	0.57	0.26	0.02
PSO3	1.12	0.42	0.03

Explanations as in Tab. 5.
Source: own study.

Based on Table 7, PSO1 treatment was identified as the most effective bioleaching condition. It resulted in the highest Au extraction and accumulation in both pellet component containing bacterial cells and in the supernatant, while leaving the lowest Au content in the tailing residue. The multielement content remaining in the residue is shown in Table 8.

Based on the multielement mass balance calculations, As remained the most abundant element in the residue across all

Table 8. Average multielement content in the general name of residue, supernatant and pellete after the second-stage bioleaching experiment

Element	Content (ppm)		
	PSO1	PSO2	PSO3
In residue			
Ag	0.241	0.244	0.244
As	864.786	789.463	1,131.489
Cu	17.883	14.341	16.302
Sb	157.728	201.114	124.904
In supernatant			
Ag	0.244	0.244	0.244
As	2.504	1.055	9.666
Cu	<0.002	0.001	0.005
Sb	0.185	0.210	0.390
In pellet			
Ag	0.244	0.244	0.280
As	1,416.018	968,433	1,452.629
Cu	25.770	14.780	21.834
Sb	190.929	146.948	142.076

Explanations as in Tab. 5.
Source: own study.

treatments after the bioleaching experiment. This was likely due to the refractory nature of Au in arsenic pyrite, which is commonly found in Carlin-type gold deposits (Hofstra and Cline, 2020). The gold deposits at the Ratatotok site are classified as Carlin-type. In addition to As, gold mine tailings also contain other mineral elements bound to sulphide compounds that may still be recoverable (Chingwaru, Heyden von der and Tadie, 2023; Lemos *et al.*, 2023). For this reason, the content of Ag, Cu and Sb also remained relatively high following the bioleaching process (Tabs. 4 and 8).

COMPARISON OF GOLD CONTENT BETWEEN TREATMENTS AFTER BIOLEACHING

The level of significance in Au content after bioleaching treatments was assessed using the ANOVA test. Based on the statistical test, the Au content in the supernatants, pellets, and residues in the first-stage bioleaching experiment did not show a significant difference between treatments ($p > 0.05$), namely between room temperature (25°C) and 50°C. However, at a temperature of 50°C, the Au content in the pellet containing bacterial cells tended to be higher than that at room temperature (25°C). The Au content in the residue in the second-stage bioleaching experiment showed a significant difference between treatments ($p < 0.05$), while the Au content in the pellet and supernatant components did not show a significant difference between treatments ($p > 0.05$), namely the Au content in the tailings was 0.58 ppm (code PSO1), 0.63 ppm (code PSO2) and 0.97 ppm (code PSO3).

DISCUSSION

Bioleaching treatment produces three distinct components through a 2-fold filtering process: first using 400-mesh filter paper, followed by 600-mesh filter paper. The three components were pellet containing *Bacillus cereus* RTKS bacterial cells, tailing residue, and supernatant which was a mixture of bacterial growth media liquids. This separation into three components is necessary for evaluating metal recovery in solid materials by bacterial cells (Rendón-Castrillón *et al*., 2023). Gold extraction was found to be higher in the supernatant than in the pellet (Tab. 3, 6). This finding aligns with the research by Kudpeng, Thayanukul, and Thiravetyan (2021), which reported that the supernatant (without the presence of bacterial cells) was more effective at leaching gold from ore than direct leaching by bacteria. This is likely due to the toxic effect of metals on bacterial cells, which can inhibit Au absorption. In contrast, the supernatant contains organic acids produced by bacteria, which play a crucial role in gold bioleaching.

Organic acids produced by microorganisms can damage the surface and internal structure of the ore leading to decomposition of SiO_2 and other surface compounds. This process enhances metal recovery by increasing ore's porosity and permeability (Wang *et al*., 2024).

Furthermore, Faraji *et al*. (2021) stated that the loss of gold in the gold recovery process by cyanide-producing bacteria can occur due to the biosorption of Au onto bacterial cells. Therefore, it is important to evaluate cell-free biocyanidation media to avoid biosorption of Au onto the surface of bacterial cells and reduce Au losses during recovery.

Bacillus is a cyanide-producing bacteria, and the higher the cyanide production, the greater the percentage of Au that can be recovered from ore (Aminian-Dehkordi *et al*., 2020).The most effective treatment was evaluated based on the highest percentage of Au extraction and the greatest bioaccumulation of Au in bacterial cells (Kudpeng, Thayanukul and Thiravetyan, 2021). Although the Au content in the three components after the first-stage bioleaching treatment did not show significant differences ($p > 0.05$), the Au content in the pellet tended to be higher (Tab. 2). Moreover, the extraction of Au in the pellet and tailing residue was much higher at 50°C (Tab. 3), and the loss rate at this temperature showed the lowest average value. Therefore, the second stage bioleaching treatment, which tested variations in tailing Au content, was carried out at 50°C. An increase in temperature can increase metal leaching efficiency in the bioleaching process up to a certain extent (Srichandan *et al*., 2019). A previous study (Altinkaya *et al*., 2018) reported that bioleaching of Au, Cu, Fe, Ni, Co, and Zn at acidic pH by acidophilic bacteria was effectively maintained at 32°C.

Apart from pH and temperature, the presence of other elements can also influence the effectiveness of bioleaching by microorganisms, either increasing or decreasing the effectiveness depending on the element type. For example, Fe^{3+} and Al^{3-} can inhibit the growth of bioleaching agent microorganisms and reduce the effectiveness of metal bioleaching, but F^{-} has the opposite effect (Shang *et al*., 2015). The addition of macroparticle elements can also increase the efficiency of metal extraction by microorganisms in bio-ore pellets (Li *et al*., 2022). Moreover, the addition of iodide-iodine mixtures can increase the ability of bacteria to dissolve gold from ore which mostly consists of gold, pyrite, galena and chalcopyrite (Khaing, Sugai and Sasaki, 2019).

High As content can inhibit bacterial growth (Breed, Dempers and Hansford, 2000), thereby reducing the ability of bacteria to extract and accumulate Au during the bioleaching process. The As element is commonly associated with Au in Carlin-type gold deposits (Hofstra and Cline, 2020), while the gold deposits at the Ratatotok site from which the tailings were sourced. Therefore, in the treatment with variations in Au content in the tailings, the PSO3 treatment with the highest Au content produced the residue with the highest Au content and the highest As content (Tab. 8). However, based on the ANOVA test ($p > 0.05$), the Au content in the pellet and supernatant components after bioleaching did not show significant differences. This indicated that the increase in Au content in the tailings to the PSO3 level did not affect the performance of Au accumulation by *Bacillus cereus* RTKS. This is because there was no difference of the bacterial cells number used in the PSO1, PSO2 and PSO3 treatments. The number of bacterial cells affects the percentage of Au that can be recovered from the ore, the higher bacterial cells number, the higher the percentage of Au that can be recovered (El-Sayed *et al*., 2021).

CONCLUSIONS

The results showed that a temperature of 50°C was more favourable for gold bioleaching by *Bacillus cereus* RTKS compared to room temperature (25°C). This was demonstrated by a higher percentage of Au extraction and greater Au accumulation in the pellet after bioleaching than at the 25°C treatment. In addition, the loss rate at 50°C was the lowest. Therefore, a temperature of 50°C was then used for the 2nd stage bioleaching to text the variations in the Au content in the tailings. The results of the 2nd stage bioleaching showed that increasing the tailing concentration as indicated by increasing the Au content in the tailings did not increase the capacity of bacteria to recover Au from the tailings. This was indicated by no significant difference in Au content in the pellet between treatments. However, there was a significant difference in Au content in the residue, which was significantly higher in the bioleaching treatment with the highest Au content of tailings. This result was possible because there was no difference in the number of bacterial cells between treatments, while the number of cells influenced the ability of bioaccumulation and metal recovery by bacteria. The presence of other elements, especially As, was also a factor that inhibited gold recovery by bacteria, where in treatments with higher Au tailing content, the As content was also higher.

ACKNOWLEDGEMENTS

The authors would like to thank the Directorate General of Higher Education for funding this research. We are also grateful to PT Sumber Energi Jaya (PT SEJ) for their support and cooperation during the tailing sampling at the mine site, as well as for providing access to laboratory facilities for preparing samples. Special thanks are also extended to Rania Nur Farida and Melinda Tri Hartanti, for their assistance with laboratory work in the Microbiology Laboratory at Universitas Negeri Yogyakarta.

FUNDING

This research was funded by the Directorate General of Higher Education of Indonesia through the 2024 Research Grant of "Penelitian Fundamental Reguler".

CONFLICT OF INTERESTS

All authors declare that they have no conflict of interests.

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