The Potential of \textit{Bacillus cereus} S1 as an Environmentally Friendly Bioaccumulator of Gold Nanoparticle Waste

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Abstract. Gold (Au) is one of the metals that is widely used for jewellery and electroplating. Although in the nanoparticle form, the waste that produced still has high economic value. Since its belong to heavy metals, gold nanoparticle waste need to be removed even in the very low concentration. One of the alternatives is utilized gold-resistant bacteria, namely \textit{Bacillus cereus} S1. The purpose of our study was to determine the ability of \textit{B. cereus} S1 to accumulates gold. Reconfirmation test of \textit{B. cereus} S1 gold resistant ability was figured out using Minimal Salt Medium (MSM), 2\% glucose and 0.1 ppm gold. The bioaccumulation process was used 1 ppm, 5 ppm, and 10 ppm concentration of gold; and the incubation periods were 6, 12, and 24 hours. Gold bioaccumulation performed by \textit{B. cereus} S1 was measured using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-EOS). The results showed that the longer incubation periods of \textit{B. cereus} S1, the higher gold concentration will be accumulated at exposure concentration 1 ppm, 5 ppm and 10 ppm. \textit{B. cereus} S1 showed good viability after 24 hours metal exposure.

Keywords: \textit{Bacillus cereus} S1, Bioaccumulation, Gold, Resistance.

1. Introduction

Gold or Aurum (Au) is a metal that possess high economic value and belongs to precious metals group as well as heavy metals [1]. Gold carries toxic effect if it accumulates in living organisms. Gold accumulation of 0.8 mg/L will affect \textit{Danio rerio} (zebra fish) genetic material [2]. Several methods have been carried out to overcome gold waste pollution both in physical and chemical treatment. For chemical treatment, solvents such as halides, cyanides, and thiosulfates were used for hydrometallurgical processes while physical treatment utilizes high temperature pyrometallurgical processes. However, all treatments above caused other toxicity problems since Hg, Fe, Al, HNO$_3$, CN and HCl will be released to the environment [3].

Renewable biological alternatives that can be applied to overcome gold contamination in the environment by using bioaccumulator agents, termed gold-resistant bacteria. \textit{Cupriavidus metallidurans} is able to accumulate gold through Au (III) reduction into Au (0) [4]. The \textit{Bacillus} genera is also capable to accumulates gold from HAuCl$_4$ up to 306 $\mu$mol / gram dry cell [5]. In previous studies, \textit{Bacillus cereus} S1 was resistant to heavy metals such as Hg, Cd, Pb, Cu [6], also reported that \textit{B. cereus} S1 is capable to reduce Cr (IV) into Cr (III)[7]. However the ability of isolate to accumulate gold is remain unexplored. Therefore, the aim this study was to examine the resistance and bioaccumulation ability of \textit{Bacillus cereus} S1 against gold.
2. Material and Methods

2.1. Viability Test on Acidic pH
The isolate used in this study is *Bacillus cereus* S1 [8]. The isolate resistance test to acidic pH was carried out to determine isolate growth ability in acidic environment, since HAuCl₄ solution known to be acidic [5]. The test was performed by making nutrient broth medium with varies pH set, as follows 3; 4; 5 and 6. pH adjustment by adding NaOH or HCl until desired pH was reached. Bacterial culture with 10⁶ density added to the medium as much as 5 ml / 200 mL medium, then incubated at room temperature for 12 hours. Optical density was measured using Genesys UV-Vis Spectrophotometer ThermoFischer® US (λ 600 nm) in order to find out isolate growth pattern [9].

2.2. Isolate Resistance Test on Gold (Au)
Resistance test was conducted to determine the ability of *B. cereus* S1 to grow in Au metal containing medium by using HAuCl₄ as gold compound [9]. The modified MSM medium (MSMG) contain (per liter) MgSO₄ 0.2 gr, CaCl₂ 0.02 gr, KH₂PO₄ 1 gr, K₂HPO₄ 1 gr, NH₄NO₃ 1 gr, FeCl₃ 0.05 gr and Glucose 2%. MSMG-agar medium made up by adding 1.5 gr agar / 100 mL. Resistance test was performed on MSMG-agar slant media which contains 0.1 ppm HAuCl₄, incubated at room temperature for 3 x 24 hours [6]. Isolate that able to grow was classified as gold resistant isolate.

2.3. Gold Bioaccumulation Test

2.3.1. H AuCl₄ Exposure. Bioaccumulation test was performed using liquid MSMG-HAuCl₄. As much as 10 mL bacterial culture (12 hours) with 10⁶ cell density was added to 90 mL MSMG-HAuCl₄ with concentration of 1 ppm, 5 ppm and 10 ppm. Furthermore, the treatment culture was incubated for 6 hours, 12 hours and 24 hours in the rotary shaker (100 rpm).

2.3.2. Measurement of H AuCl₄ Bioaccumulation. Cultures that have been exposed to HAuCl₄ was added with 10 drops of HNO₃, and then heated to 100º C for 10 minutes. Cultures then centrifuged (4000 rpm) for 10 minutes. Natant was put in a dark bottle containing 100 ml aquabides. Gold extraction from bacterial cells was carried out using sonicator (600 watts, 50% amplitude) at 4ºC for 60 seconds [10]. The gold concentration was measured by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) with a wavelength of 242.8 nm [11].

2.4. Viability Test after H AuCl₄ Exposure
The objective of viability test was to determine viability of isolates after exposure to HAuCl₄. The total of 100 µL of H AuCl₄ exposed isolates were inoculated on nutrient agar medium using the pour plate method. Growing colonies isolates were able to survive are viable to the exposure of H AuCl₄, viability level was calculated using the CFU (colony forming unit) method.

3. Result and Discussions

3.1. Isolate Viability on Acidic pH
At incubation time of 0 - 12 hours, *B. cereus* S1 showed the better growth pattern at pH 5 and pH 6 compared to pH 3 and pH 4. Growth patterns at pH 3 and pH 4 were relatively flat with optical density value 0.2 - 0.4. While growth pattern at pH 5 and pH 6 showed normal growth pattern, which consist of lag (adaptation) phase, logarithmic, stationary and death, with OD range between 0.3 - 1.2 (Figure 1). The growth of *B. cereus* S1 in pH 3 and 4 is very low and tends to be stagnant compared to its growth in pH 5 and 6. This can be occurred due bacteria were gripped by low pH and carried out survival strategy without fission.
Figure 1. The growth pattern of isolate *B. cereus* S1 in liquid MSMG medium with pH set 3-6. Bacteria isolate grow well in pH 5 and 6 which indicated from OD value between 0.4-1.2.

According to [12], *B. cereus* was able to survive in low pH because it has a sporulation mechanism, so that, fission will be suppressed and formed endospores. Under acidic conditions, the proton (H⁺) concentration outside the cell was higher than inside the cell, so that H⁺ diffused into the cell. Excessive accumulation of H⁺ caused clogged porins, inhibited protons motive force, and impaired membrane permeability [13]. The growth of isolates at pH 6 showed better growth pattern compared to other pH treatment since the pH on the medium was almost the same as in the cytoplasm, so that pH homeostatic occurred between intracellular and extracellular.

*B. cereus* S1 which cultivated at pH 6 shows good viability indicated by its growth pattern (Figure 1). For further experiments, pH of medium which used in this study was adjusted to 6. According to [9], at medium with pH 6 HAuCl₄ will turned to Au(OH)₄, if the isolate is placed in a solution containing Au(OH)₄ then the ligand will be formed between bacteria cells and Au.

3.2. Bacillus cereus S1 Resistance on Gold

After incubation for 24 hours on MSMG slant medium containing 0.1 ppm HAuCl₄, *B. cereus* S1 was resistant to Au metal. Resistance is characterized by the growth of isolates in the medium (Figure 2). The concentration of 0.1 ppm HAuCl₄ was used as resistance adaptation considering that if in the low concentrations there is no growth from isolates means the isolates did not able to resist with Au metals exposure.

*B. cereus* S1 is isolate that has been examined for resistance to several heavy metals, such as Hg, Cd, Pb, Cu [6]. *B. cereus* S1 is also resistant to Au metals where Au metal is also a heavy metal since it has a specific gravity of 19.3g/ cm³. According to [14], bacterial resistance to Au metal by binding Au to the plasma membrane. Then it will be reduced to nanoparticles in the cytoplasm, so that nontoxic metal ions are transported out from cytoplasm. This transport mechanism aims to maintain equilibrium between the cytoplasm and cell membrane.
Figure 2. B. cereus S1 colony which grow on (a) medium without Au, (b) medium containing 0.1 ppm of Au (Red arrows pointed at the growing colony on agar slant).

For enzymatic mechanism, there are several genes that are responsible for gold resistance, termed gig gene (gold induced gene) which consists of GolT, GolB, GolS, and gesABC. GolT is transmembrane ATPase. GolB is an Au binding protein in the cytoplasm heading to NADPH reductase. GolS is an enzyme that regulates transcription of gesABC, golTS, and golB. gesABC act as protein efflux [15].

3.3. Gold Metal Bioaccumulation Performed by Bacillus cereus S1

The longer incubation period, the more Au bioaccumulation by B. cereus S1 increases in each treatment concentration. The highest Au bioaccumulation occurred at 10 ppm concentration with 24 hours incubation, while the lowest bioaccumulation occurred at a concentration 1 ppm which was incubated for 6 hours (Figures 3 and 4). Regarding [14], the bioaccumulation of gold by bacteria cells will increase with the length of incubation time.

Figure 3. Gold bioaccumulation performed by B. cereus S1 in liquid MSMG (pH 6). The highest accumulation occurred at 10 ppm with 24 hours incubation.
Figure 4. Percentage (%) of gold accumulation performed by \textit{B. cereus} S1 in liquid MSMG (pH 6). The highest gold accumulation efficiency occurred in 5 ppm concentration treatment in 24 hours incubation with percentage value 34.52%.

\textit{B. cereus} S1 accumulates gold more efficiently at concentrations of 1 ppm and 5 ppm than 10 ppm, with value 31.9% and 34.52% respectively (Figure 4). This showed that gold in 10 ppm concentrations was toxic to \textit{B. cereus} S1. According to [16], gold at a concentration below 5 ppm has relatively small toxicity effect. According to [17], bacterial cell membranes have functional groups that can provide free electron pairs for heavy metals. The amine and hydroxyl functional groups in the membrane act as free electron donors which react with heavy metal cations to form ligand bonds.

3.4. \textit{Bacillus cereus} S1 Viability after Gold Exposure

After exposure to gold for 24 hours, \textit{B. cereus} S1 showed good viability which indicated by the number of growing colonies on nutrient agar medium without gold. At the incubation time of 6 hours the number of CFU \textit{B. cereus} S1 was less than the 12\textsuperscript{th} and 24\textsuperscript{th} hours, this was related to cell growth which influenced by the incubation time (Figure 5).

Figure 5. \textit{B. cereus} S1 viability after gold metal exposure. In all gold treatment concentration, CFU increased along with incubation periods. The highest CFU number occurred at 24 hours incubation.

Based on the growth pattern of \textit{B. cereus} S1 in Figure 1, this viability test is in accordance with the growth pattern which the log phase begins after 6 hours of incubation. \textit{B. cereus} S1 was able to survive after gold exposure up to 10 ppm. According to [14], the death of several bacterial cells due to exposure to gold can occur at concentration of 80 ppm.
4. Conclusion

*B. cereus* S1 is resistant on gold up to a concentration of 10 ppm which is indicated by its viability after 24 hours exposure. The highest bioaccumulation occurred at 24 hours incubation periods on all concentration exposures, with the highest % accumulation at 5 ppm concentration with percentage value 34.52%.

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