Extraction of gold (Au) particles from sea water by *Delftia Acidovorans* microbes

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Abstract. Gold-mining activities have been an issue, especially when it involves in contamination of chemicals, for example arsenic and mercury. However, despite of these hazards, gold-mining activities are still being conducted. This is because the gold is worth, regardless of the problems. Gold-mining, as known needs a very large area of land, or site plant. Vast amount of labor force, mechanical force and fund are a must in order for the mining process to be continued. High demand of gold, made gold-mining industries as ones of the most profitable industries in the world. Thus, this has encouraged another alternative way to extract gold. At the mining site, researchers found that biomineralization of gold by *Delftia acidovorans* can be conducted. How it is done still cannot be understood. It is said that the bacteria secretes secondary metabolites, Delftibactin as a defensive mechanism against the toxicity of the soluble gold. Researchers try to find another source of elemental gold besides of the earth’s core. The options are either lava of a volcano or ocean. Here, the focus is seawater. The problem of seawater is that its composition still not yet to be proved. Dissolve gold existed as gold chloride in seawater, but in a very small amount. So, the gold separation should be focused, in order to make this process to be a successful one. Factors such as depth, climate, region, temperature need to be considered. If this difference affecting the separating process, standardized seawater composition have to be proposed.

1. Introduction

Seawater is a complex mixture of 96.5% of water and 2.5% of salts and other smaller substances, which are either organic, inorganic materials or a few atmospheric gases [1]. 70% of Earth’s surface is covered with seawater, and by this enormous composition of the foundation, this automatically marks seawater as the biggest source for gold metal [2]. However, in reality, there is only a small amount of gold existed in seawater. In fact, gold, Au is the least inorganic compound that presents in seawater, other than its periodic table neighbors such as Ag, Pd and Pt.

In seawater, gold, Au exists in its most stabilized form that is gold chloride, AuCl$_3^-$ [3]. This soluble form of gold is very toxic, and it harms human being. However, in spite of its toxicity, scientists have found that there is a superman-strength bacteria can survive in that toxicity condition. This bacteria is a prokaryote bacteria named *Delftia acidovorans*. *D. Acidovorans* is said to be resisting the toxic effects of this heavy metal. It deposited around the gold nuggets as a bacterial film, which make it available for redepositing in purer form somewhere else in the mine [4]. Therefore, researchers started to focus on this bacteria in order to develop other ways of extracting gold that are much cheaper and safe compare to the gold-mining process [5].
2. Methodology

2.1 Materials

2.1.1 Raw materials preparation
There will be eight types of raw materials used in this research. The materials are *Delftia acidovorans* strains, Acidovorax complex medium (ACM), Delftibactin, gold chloride (AuCl₃), seawater, 0.5% Agarose solution, double distilled water, and calcium carbonate.

2.1.2 Bacteria cultures
*Delftia acidovorans* strains will be cultured on Acidovorax complex medium (ACM) plates at 30°C.

2.2 Methods

2.2.1 Gold biomineralization
Gold biomineralization will be done by precipitation of gold on agar plate. *Delftia acidovorans* will be streaked onto a chelex-treated (deferrated) Acidovorax Complex Medium (ACM) agar plate. The bacteria will be grown for three days at 30°C. The culture will be centrifuged and resuspended in 5 mL sterilized ddH₂O. During the research, gold biomineralization step will be the main focus. There will be two factors involves; A (first factor), the concentration of gold chloride, and B (second factor), the concentration of Delftibactin, and C (third factor), the volume of seawater.

2.2.2 Factor A: Concentration of Gold Chloride
The assays will be set up as in 50 μL of ddH₂O containing 0.2 mM, 0.4 mM, 0.6 mM, 0.8 mM, 1.0 mM of Gold Chloride. Each sample will be added with 50 μL of 1.0 mM Delftibactin. Controls containing only water, which is No- Delftibactin and No- AuCl₃ will be added as a sample. The mixture will be incubated for 30 minutes in room temperature. Then, 100 μL of concentrated bacteria culture will be added to each sample. Incubate again for 30 minutes in room temperature. The mixture will be then incubated for 48 hours at 30°C. However, the absorbance reading will be recorded at first 120 minutes.

2.2.3 Factor B: Concentration of Delftibactin
The assays will be set up as in 50 μL of ddH₂O containing 0.2 mM, 0.4 mM, 0.6 mM, 0.8 mM, 1.0 mM of Delftibactin. Controls containing only water, which is No- Delftibactin and No- AuCl₃ will be added as a sample. Then, 100 μL of concentrated bacteria culture will be added to each sample. Incubate again for 30 minutes in room temperature. Seawater with volume of 5 liters will be introduced with 500ml of 5.0 M of Calcium Carbonate. The mixture will be allowed to settle, and sludges should be formed after several hour. The times normally vary based on the varying contents of said seawater. But, in this project, it will be limited to 48 hours. After that the water will be siphoned out, and sludges will be collected. Weight the wet weight of the sludge. The sludges are then introduced to the assays samples with different concentration of Delftibactin. The mixture is then incubated for 48 hours. However, the absorbance reading will be recorded at first 120 minutes.

2.2.4 Factor C: Volume of Seawater
The assays will be set up as in 50 μL of ddH₂O 1.0 mM of Delftibactin. Controls containing only water, which is No- Delftibactin and No- AuCl₃ will be added as a sample. Then, 100 μL of concentrated bacteria culture will be added to each sample. Incubate again for 30 minutes in room temperature. Seawater with volume of 1, 5, 10, and 15 liters will be introduced with 500ml, 1000ml, 1500ml, and 2000ml respectively of 5.0 M of Calcium Carbonate. The mixture will be allowed to settle, and sludges
should be formed after several hour. The time normally varies based on the varying contents of said seawater. But, in this project, the will be limited to 48 hours. After that the water will be siphoned out, and sludges will be collected. Weight the wet weight of the sludge. The sludges is then introduced to the assays samples with different concentration of Delftibactin. The mixture is then incubated for 48 hours. However, the absorbance reading will be recorded at first 120 minutes.

2.2.5 Extraction method for gold nanoparticles
The suspension will be centrifuged and washed once with water to concentrate the precipitate. The precipitate will be resuspended in ddH₂O. Au³⁺ in aqueous solution will be monitored by periodic sampling of aliquots (0.2ml) of the suspension. Each time the sample will be diluted with deionized water. UV-VIS spectra will be recorded by the use of Spectrophotometer at absorbance value of 600nm. UV-VIS will be used to examine the size and shape controlled nanoparticles in aqueous suspensions.

3. Results and Discussion

3.1 Wet weight of sludges
The sludges were obtained from the settlement of the mixture of the seawater and calcium carbonate. From Table 1.0, we can see that the wet weight of the sludges was varied. Eventhough, there was an increment of volume on both concentration of gold chloride, volume of seawater and volume of calcium carbonate added, the weight of the sludges diverged. Adding calcium carbonate in seawater was one of the assimilation of formation of calcrete on soils (as it occurs in nature). The vast different in wet weight was mainly due to a different consistency in the seawater composition. This different composition would react differently, and thus amount of sludges formed showed a discrepancy.

| Concentration of Delftibatin, mM | Wet weight of sludges,g |
|---------------------------------|------------------------|
| 0.2                             | 84.6                   |
| 0.4                             | 72.5                   |
| 0.6                             | 80.3                   |
| 0.8                             | 116.2                  |
| 1.0                             | 94.1                   |

| Volume of Seawater, liters      | Wet weight of sludges,g |
|---------------------------------|------------------------|
| 1.0                             | 486.9                  |
| 5.0                             | 725.3                  |
| 10.0                            | 1068.4                 |
| 15.0                            | 981.5                  |

3.2 Factor A: Concentration of Gold Chloride

Controlled Variable: 1.0 mM Delftibactin
During the incubation process, gold element was separated from chloride and forming a colloidal. From Table 2.0, which shows weight of gold particles extracted, we can see that the number of particles existed in the sample affecting the reading of absorbance. Thus, we can
concluded that at 0.2 mM concentration, it shows the lowest weight, which is proven on Table 2.0

| Concentration of Gold Chloride, mM | Incubation Time, min | UV-VIS Reading(600nm), mm | Final Weight, g |
|-----------------------------------|----------------------|---------------------------|-----------------|
| 0.2                               | 0                    | 0.038                     | 0.183           |
|                                   | 5                    | 0.033                     |                 |
|                                   | 15                   | 0.037                     |                 |
|                                   | 30                   | 0.071                     |                 |
|                                   | 60                   | 0.085                     |                 |
|                                   | 120                  | 0.102                     |                 |
| 0.4                               | 0                    | 0.044                     | 0.267           |
|                                   | 5                    | 0.041                     |                 |
|                                   | 15                   | 0.041                     |                 |
|                                   | 30                   | 0.096                     |                 |
|                                   | 60                   | 0.134                     |                 |
|                                   | 120                  | 0.221                     |                 |
| 0.6                               | 0                    | 0.047                     | 0.445           |
|                                   | 5                    | 0.058                     |                 |
|                                   | 15                   | 0.074                     |                 |
|                                   | 30                   | 0.159                     |                 |
|                                   | 60                   | 0.193                     |                 |
|                                   | 120                  | 0.282                     |                 |
| 0.8                               | 0                    | 0.050                     | 0.522           |
|                                   | 5                    | 0.059                     |                 |
|                                   | 15                   | 0.071                     |                 |
|                                   | 30                   | 0.204                     |                 |
|                                   | 60                   | 0.286                     |                 |
|                                   | 120                  | 0.283                     |                 |
| 1.0                               | 0                    | 0.078                     | 0.764           |
|                                   | 5                    | 0.104                     |                 |
|                                   | 15                   | 0.126                     |                 |
|                                   | 30                   | 0.232                     |                 |
|                                   | 60                   | 0.255                     |                 |
|                                   | 120                  | 0.308                     |                 |

3.3 Factor B: Concentration of Delftibactin
Controlled Variable: 5 liters Seawater
From Table 3.0, it is observed that the absorbance value increased as the concentration of Delftibactin increased. During the incubation process, gold element was separated from the sludges. At 1.0 mM concentration, the absorbance value shows its highest value. Factor B showed to produce a lesser amount of gold final weight as compared to Factor A. This shows that data in Factor A show a wider range of light scattered compared to Factor B since there is more number of gold particles existed in the samples.

| Concentration of Delftibactin, mM | Incubation Time, min | UV-VIS Reading(600nm), nm | Final Weight, g |
|-----------------------------------|----------------------|---------------------------|-----------------|
| 0.2                               | 0                    | 0.152                     | 0.000           |
|                                   | 5                    | 0.158                     |                 |
|                                   | 15                   | 0.172                     |                 |
|                                   | 30                   | 0.194                     |                 |
|                                   | 60                   | 0.252                     |                 |
|                                   | 120                  | 0.280                     |                 |
| 0.4                               | 0                    | 0.182                     | 0.0016          |
|                                   | 5                    | 0.186                     |                 |
|                                   | 15                   | 0.204                     |                 |
|                                   | 30                   | 0.211                     |                 |
|                                   | 60                   | 0.282                     |                 |
|                                   | 120                  | 0.307                     |                 |
| 0.6                               | 0                    | 0.215                     | 0.0000          |
|                                   | 5                    | 0.230                     |                 |
|                                   | 15                   | 0.256                     |                 |
|                                   | 30                   | 0.294                     |                 |
|                                   | 60                   | 0.325                     |                 |
|                                   | 120                  | 0.372                     |                 |
| 0.8                               | 0                    | 0.240                     | 0.0000          |
|                                   | 5                    | 0.247                     |                 |
|                                   | 15                   | 0.290                     |                 |
|                                   | 30                   | 0.342                     |                 |
|                                   | 60                   | 0.385                     |                 |
|                                   | 120                  | 0.403                     |                 |
| 1.0                               | 0                    | 0.301                     | 0.0045          |
|                                   | 5                    | 0.320                     |                 |
|                                   | 15                   | 0.354                     |                 |
3.4 Factor C: Volume of Seawater

**Controlled Variable: 1.0 mM Concentration of Delftibactin**

From Table 4.0, it is observed that the absorbance value increased as the volume of seawater increased. During the incubation process, gold element was separated from the sludges. At 20 liters of seawater, the absorbance values show its highest value. When comparing with Table 3.0, which shows no gold particles were extracted, we can see that the number of particles exists in the sample was not affecting the reading of absorbance. But instead, the composition or mostly the salinity of seawater affecting the reading of absorbance.

| Volume of Seawater, liters | Incubation Time, min | UV-VIS Reading(600nm), nm | Final Weight, g |
|----------------------------|----------------------|---------------------------|----------------|
| 1.0                        | 0                    | 0.1402                    | 0.0000         |
|                            | 5                    | 0.1486                    |                |
|                            | 15                   | 0.1830                    |                |
|                            | 30                   | 0.2361                    |                |
|                            | 60                   | 0.2522                    |                |
|                            | 120                  | 0.2818                    |                |
| 5.0                        | 0                    | 0.3005                    | 0.0028         |
|                            | 5                    | 0.3188                    |                |
|                            | 15                   | 0.3561                    |                |
|                            | 30                   | 0.3538                    |                |
|                            | 60                   | 0.3684                    |                |
|                            | 120                  | 0.3820                    |                |
| 10.0                       | 0                    | 0.3205                    | 0.0004         |
|                            | 5                    | 0.3372                    |                |
|                            | 15                   | 0.3584                    |                |
|                            | 30                   | 0.3804                    |                |
|                            | 60                   | 0.3887                    |                |
|                            | 120                  | 0.3951                    |                |
| 15.0                       | 0                    | 0.3258                    | 0.0000         |
|                            | 5                    | 0.3294                    |                |
|                            | 15                   | 0.3406                    |                |
|                            | 30                   | 0.3784                    |                |
|                            | 60                   | 0.3902                    |                |
|                            | 120                  | 0.4050                    |                |
3.5 Final Weight of Gold Particles
Table 5.0 shows the comparison of final weight of gold particles between gold chloride and seawater. As gold chloride is the stable form of gold particles in nature, we can directly assume that the extraction from gold chloride will definitely produce the highest number of gold particles. As mentioned previously, even though there is a constant amount of gold in seawater, but in different collection of seawater samples there is no guarantee there is gold present in the samples. This explains why most of the test extracted no gold despite the increment in seawater volume used.

| Time | Gold Chloride | Seawater |
|------|---------------|----------|
| 0    | 0.3108        | 0.0000   |
| 5    | 0.3237        |          |
| 15   | 0.3409        |          |
| 30   | 0.3552        |          |
| 60   | 0.3885        |          |

4. Conclusion
The reduction of Au\(^{3+}\) ions by Delftia sp. resulted in the formation of stable particles from gold chloride solution. However, the purpose of this main project is to be able to extract gold particles from seawater. The objectives were successfully accomplished even though the amount of gold particles extracted was almost to non-existent. In spite of this, the success in extracting this small amount of gold particles can proved that it is possible to obtain gold from the vast amount of seawater. Even though it is possible, the small amount of particles make this project impractical to be done in industries level. The expenditure to start the project was expensive, but the recovery of gold from seawater unable to compensate the start-up modal. Engineered microbes or improved equipment might help in the extraction in the future.

5. References
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