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Microbial recovery of gold from neutral and acidic solutions by the baker's yeast *Saccharomyces cerevisiae*

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ABSTRACT

An environmentally friendly method using the baker's yeast *Saccharomyces cerevisiae* was developed to recover soluble Au(III) from neutral and acidic solutions at room temperature. Resting cells of *S. cerevisiae* were able to reduce aqueous Au(III) ions in $HAuCl_4$ solution to metallic Au(0) at pH 7.0 within 120 min when formate was provided as the electron donor under anaerobic conditions. Gold nanoparticles were deposited on the cell surface of *S. cerevisiae*. The *S. cerevisiae* cells were also applicable to a bio-material for adsorbing aqueous Au(III) ions from $HAuCl_4$ solution at pH 1.0 under air atmosphere. When processing the aqua regia leachate of electronic waste (Central Processing Units, CPU), the *S. cerevisiae* cells were able to rapidly and selectively collect aqueous Au(III) ions from the aqua regia leachate at pH 1.2 within 10 min. Our proposed microbial methods enable the rapid and efficient recovery of gold over the pH range 1.0 to 7.0.

1. Introduction

Recycling of gold from secondary sources is increasing in importance because of gradual depletion of natural mineral resources and increased consumer demand. A number of wet chemical methods, such as crystallization and precipitation, hydrolysis, solvent extraction, adsorption, and metal reduction, have been used to separate and purify gold from various liquids such as leach solutions of waste electrical and electronic equipment (WEEE), electroplating wastes, eluates from activated carbon and spent liquors. In general, these liquids show a wide range of gold concentrations varying from 1 to 2000 ppm (Miller et al., 1990; Flores and O'Keefe, 1995). Although conventional chemical recovery techniques remain the best methods for recovering gold from various liquids, biological recovery methods provide an attractive and eco-friendly alternative strategy, in which microorganisms are used to separate and concentrate soluble gold from dilute solutions into microbial cells at room temperature, via adsorption and reduction of soluble Au(III) ions.

Previous work has reported microbial reduction and deposition of gold under anaerobic conditions, a process known as biomineralization, by using several types of microorganisms. These microorganisms include: (i) the mesophilic Fe(III)-reducing bacterium *Shewanella algae* (Konishi et al., 2006; Konishi et al., 2007a; 2007b), (ii) anaerobic Fe (III)-reducing mesophiles and thermophiles (Kashefi et al., 2001), (iii) the mesophilic sulfate-reducing bacterium *Desulfovibrio desulfuricans* (Creamer et al., 2006; Deplanche and Macaskie, 2008), (iv) the intestinal bacterium *Escherichia coli* (Deplanche and Macaskie, 2008), (v) the actinomycetes *Rhodococcus* sp. (Ahmad et al., 2003a) and *Thermomonospora* sp. (Ahmad et al., 2003b), and (vi) the fungi *Verticillium sp* (Mukherjee et al., 2001), *Fusarium oxysporum* (Mukherjee et al., 2002) and *Aspergillus niger* (Bhambure et al., 2009). Application of fungi and actinomycetes in gold recovery has the disadvantage that the reduction of soluble Au(III) is very slow, with complete reduction taking 48–120 h. On the other hand, previous studies have indicated that in a solution of pH 7, the mesophilic Fe(III)-reducing bacterium *S. algae* was able to completely reduce 1.0 mol/m^3 Au(III) ions within 30 min with formate as the electron donor under anaerobic conditions (Konishi et al., 2006; 2007a; 2007b). Moreover, the mesophilic bacteria *D. desulfuricans* and *E. coli* were able to achieve complete reduction of 2.0 mol/m^3 Au(III) ions at three different pH values of 2, 6, or 9 for 140 min, when these bacteria were combined with H_2 as the electron donor under anaerobic conditions (Deplanche and Macaskie, 2008). However, the addition of electron donor under anaerobic conditions is obligatory for rapid reduction of soluble Au(III) by these mesophilic bacteria *S. algae*, *D. desulfuricans* and *E. coli*. For the microbial Au(III) reduction, in addition, cultures of *S. algae*, *D. desulfuricans* and *E. coli* were grown anaerobically under oxygen-free nitrogen (Caccavo et al., 1992; Deplanche and Macaskie, 2008), so that it is not easy to prepare

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the large quantities of bacterial cells necessary to recover precious metals in industrial applications. Alternatively, bacteria, algae, fungi and yeast can be applied as an inexpensive bio-materials for adsorbing soluble Au(III) in neutral and acidic solutions (Das, 2010; Ilyas and Lee, 2014; Ilyas and Ilyas, 2018), a process known as biosorption. That is because they have a cell surface consisting of biological materials containing functional groups, which are responsible for the adsorption of precious metal ions from dilute solutions. However, information is generally lacking concerning the effects of the operating conditions for the recovery rates of soluble Au(III) from neutral and acidic solutions. Although previous work investigated using the mesophilic bacteria *D. desulfuricans* (Creamer et al., 2006) and *S. algae* (Saitoh et al., 2017) to recover soluble Au(III) from spent electronic scrap leachates between pH 2 and 4, little is known about the microbial ability to efficiently recover soluble Au(III) from strongly acidic WEEE leachates at lower pH values below 2.

This paper describes the recovery of soluble Au(III) from neutral and acidic solutions by resting cells of the baker's yeast *Saccharomyces cerevisiae* at 33 °C. Baker's yeast is a safe and commercially available microorganism, so that it is easy to prepare the large quantities of *S. cerevisiae* cells necessary to recover soluble Au(III). The microbial recovery of soluble Au(III) by resting cells of *S. cerevisiae* in the presence or absence of formate as the electron donor was investigated at the extremes of pH 1.0 and 7.0. The *S. cerevisiae* cells were examined to be applicable to the selective recovery of soluble Au(III) from strongly acidic leachate of electronic waste (Central Processing Units, CPU) at pH 1.2. To our knowledge, this is the first demonstration of microbial recovery of gold from strongly acidic leachate of electronic waste at lower pH value below 2.

2. Experimental section

2.1. Yeast strain and growth conditions

S. cerevisiae (NBRC 2044) was obtained from the Biological Resource Center, NITE (National Institute of Technology and Evaluation) and grown anaerobically in Glucose-yeast-peptone medium at 33 °C and pH 7.0. After 24 h of batch inoculation, *S. cerevisiae* cells were harvested by centrifugation, re-suspended in KH_2PO_4/NaOH buffer (pH 7.0), and pelleted again by centrifugation. This washing was repeated twice.

For Au(III) reduction and deposition experiments, the washed *S. cerevisiae* cells were subsequently re-suspended in KH_2PO_4/NaOH buffer and the cell suspension was bubbled with N_2 for 10 min. On the other hand, the washed *S. cerevisiae* cells were re-suspended in distilled water and the cell suspension was immediately used in Au(III) adsorption experiments over the pH range 1 to 7. The *S. cerevisiae* cells grown at pH 7.0 were not preadapted to strongly acidic solutions before adsorption experiments at pH 1.0.

2.2. Preparation of gold solutions

The metal solution containing gold (III) ions was prepared by dissolving a known weight of $HAuCl_4$ (Kanto Chemical Co., Inc., Tokyo, Japan) in distilled water. The concentration of Au(III) was held constant at 1.88 ± 0.15 mol/m^3, and the pH of each test solution was adjusted to the required value using either KH_2PO_4/NaOH buffer (pH 7.0) or 1.0 kmol/m^3 HCl solution.

On the other hand, we obtained central processing units (CPU) that are sourced from WEEE, and then the CPU were cut and crushed into small sizes of 2–4 mm. The aqua regia leachate containing Au(III) ions was prepared by dissolving a known weight of CPU in a 50% aqua regia which was diluted with deionized water in the ratio 1:1. Above 99% of gold in the CPU was leached out in 2 h at 70 °C and atmospheric pressure, and the aqua regia leachate also contained copper, nickel and iron. The aqua regia leachate of CPU was adjusted to an optimal pH 1.0 for *S. cerevisiae* activity by adding 10.0 kmol/m^3 NaOH solution. The aqua regia leachate with pH 1.0 had the following metal concentration (in mol/m^3): Au, 1.88; Cu, 7.40; Ni, 33.8; and Fe, 53.4. In microbial recovery tests, the aqua regia leachate with pH 1.0 was mixed with *S. cerevisiae* cell suspension in the ratio 2:1.

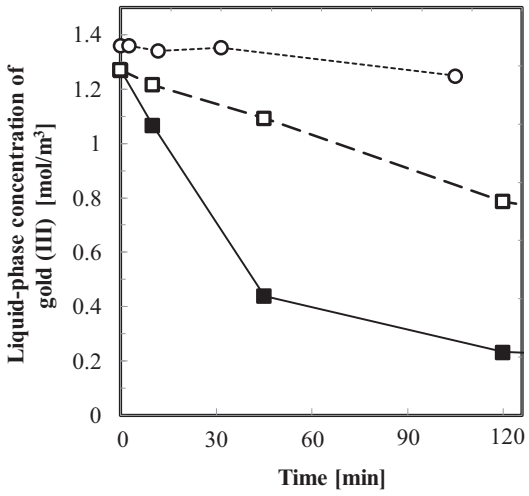

Fig. 1. Microbial reduction and adsorption of Au(III) ions in an aqueous $HAuCl_4$ solution at pH 7.0 and 33 °C under anaerobic conditions. (■) 5.0 × 10^{14} cells/m^3*S. cerevisiae* cells in the presence of 50 mol/m^3 formate; (□) 5.0 × 10^{14} cells/m^3*S. cerevisiae* cells in the absence of formate; (○) sterile control containing no *S. cerevisiae* cells in the presence of 50 mol/m^3 formate.

2.3. Experimental procedures

2.3.1. Microbial reduction and deposition of soluble Au(III)

An anaerobic glovebox was used to carry out microbial reduction experiments, as described previously (Konishi et al., 2006; 2007a, 2007b; Tamaoki et al., 2010; 2013). For a typical reduction experiment at 33 °C, 5 cm^3 of *S. cerevisiae* cell suspension was added to 10 cm^3 of aqueous $HAuCl_4$ solution with continuous stirring under N_2. The solutions were buffered at pH 7.0 with KH_2PO_4/NaOH, and the cell concentrations in the mixed solution were between 0.5 × 10^{14} and 5.0 × 10^{14} cells/m^3. The initial Au(III) concentration was held constant at 1.25 ± 0.10 mol/m^3, with 50 mol/m^3 sodium formate as the electron donor. In addition, the recovery of soluble Au(III) was also investigated in a sterile and chemical control without *S. cerevisiae* cells in the presence of formate and a control with *S. cerevisiae* cells in the absence of formate.

To follow the time course of the microbial Au(III) recovery, an aliquot of this mixture was periodically withdrawn and analyzed for gold. The concentration of gold in the liquid samples was determined by inductively-coupled plasma (ICP) spectroscopy (ICPE-9800, Shimadzu, JAPAN). The number of *S. cerevisiae* cells in the solution was counted in a Petroff-Hausser counting chamber (Hausser Scientific, Horsham, PA, USA) with a microscope (BX51, Olympus, JAPAN).

The *S. cerevisiae* cells and biogenic particles were observed by transmission electron microscopy (TEM) using a JEOL model JEM-2100F equipped with an EDX attachment (JEOL model JED-2300 T). Nano-beam electron diffraction (n-ED) was used to confirm the reductive deposition of biogenic gold nanoparticles. Samples for TEM analysis were prepared on carbon-coated copper TEM grids.

2.3.2. Microbial adsorption of soluble Au(III)

For a typical adsorption experiment at 33 °C, 5 cm^3 of *S. cerevisiae* cell suspension was mixed with either 10 cm^3 of aqueous $HAuCl_4$ solution or 10 cm^3 of the aqua regia leachate with pH 1.0 under air atmosphere, and the solution pH was adjusted using aqueous HCl