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Pretreatment of e-waste and mutation of alkali-tolerant cyanogenic bacteria promote gold biorecovery

Gayathri Natarajan, Yen-Peng Ting *

Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117585, Singapore

HIGHLIGHTS

- Pretreatment of electronic scrap promotes gold bioleaching at all pulp densities.
- Toxicity of scrap at high pulp densities decreases cyanide production.
- Unlike unadapted strains, mutated strains grow at pH 10.
- Mutated strains are alkali tolerant and improve gold recovery at pH 9.5.

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ABSTRACT

Gold was recovered from electronic scrap material (ESM) as gold–cyanide complex by *Chromobacterium violaceum* which produces cyanide as a secondary metabolite. The effect of pretreatment and mutation of alkali-tolerant bacteria was examined. Pretreatment dissolved most of the base metals, thereby reducing competition for the cyanide ion from other metals. As the pKa of HCN is 9.3, alkaline pH increases the cyanide ion concentration available for bioleaching, and the bacteria were mutated to grow at pH 9, 9.5 and 10. Results showed that at 0.5% pulp density of pretreated ESM, mutated bacteria attained gold biorecovery of 18% at pH 9, 22.5% at pH 9.5 and 19% at pH 10 while that of unadapted bacteria (at pH 7) yielded only 11% recovery. Results showed that gold bioleaching efficiency from electronic scrap was enhanced under alkaline conditions with mutated bacteria compared to bioleaching at physiological pH (around 7) of *C. violaceum*.

1. Introduction

In recent years, there has been greater awareness of the environmental problems associated with electronic waste (e-waste) disposal as consumption of electronic products and devices continues to increase (Gramatyka et al., 2007). The alarming rate of increase in e-waste may be attributed to the shorter lifecycles and the increasing demand for electrical and electronic goods. For instance, the lifespan of central processing units in computers dropped from 4–6 years in 1997 to 2 years in 2005 (Ramesh Babu et al., 2007). Mobile phones have a lifespan of less than 2 years in developed countries (Greenpeace, 2005). When the millions of computers and other electronic devices around the world become obsolete yearly, they leave behind lead, cadmium, mercury and other hazardous wastes.

EPA recently reported that over 3.19 million tons of e-waste was discarded in U.S alone and e-waste has been identified as the fastest growing component of solid municipal waste stream. Only 18% was recycled and the remaining was incinerated or landfilled (ETBC, 2012). Landfilling poses challenges, due to scarcity of landfill space as well as the concern for pollution caused by leaching of toxic heavy metals into the environment through groundwater and rainwater. Another driving force for recycling of e-waste is the recovery of precious metals. Compared with natural gold ores which has around 0.5–13.5 g gold per ton (Korte et al., 2000; Pham and Ting, 2009), electronic scrap material (ESM) has significantly higher gold content at around 10–10,000 grams gold per ton (Cui and Zhang, 2008; Pham and Ting, 2009), making it a viable alternative and more economic source of gold compared to natural ores. Indeed, these solid wastes may be considered as 'artificial ores' (Krebs et al., 1997) and can serve as secondary raw materials and reduce the demand for primary mineral resources. With increasing gold demand and depleting natural ores, there is an imperative to discover more alternative sources of gold, such as e-waste.

Bioleaching is a biohydrometallurgical process used for the extraction of minerals from their ores. It has been gaining popularity in the mining industry as a clean technology with lower operating cost and energy demand, compared to conventional technology such as pyrometallurgy and hydrometallurgy

* Corresponding author. Tel.: +(65) 6516 2190; fax: +(65) 6779 1936.
E-mail address: chetyp@nus.edu.sg (Y.-P. Ting).

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(Bosecker, 1997; Cui and Zhang, 2008; Krebs et al., 1997). In this study, *Chromobacterium violaceum*, which produces hydrogen cyanide (as a secondary metabolite) was used for bioleaching of gold from e-waste. Cyanide production by *C. violaceum* typically occurs for a short period at the early stationary phase, forming cyanide ion (CN^-) and the non-dissociated form of hydrocyanic acid (HCN). At physiological pH, cyanide is present mainly as hydrogen cyanide (pKa 9.3). Towards the late stationary and death phase, *C. violaceum* detoxifies cyanide by converting it to β-cyanoalanine (Kita et al., 2006).

Cyanogenic microorganisms form water soluble metal cyanide complexes from metal-containing solids such as printed circuit board scrap (Faramarzi et al., 2004). The process of gold cyanide complex formation in the presence of oxygen, known as gold cyanidation, is summarized in Elsner's equation (Kita et al., 2006)

Cyanogenic microorganisms mobilize metals under alkaline conditions, in contrast to acidophilic bacteria commonly used in bioleaching of heavy metals from solid waste (Brandl et al., 2008). As the pKa of HCN is 9.3, conducting the gold dissolution reaction under alkaline condition increases the total cyanide ions available for bioleaching. The challenge for such a reaction is bacterial growth under alkaline condition.

The objective of this work is to examine enhancement of bioleaching of gold from e-waste using *C. violaceum*. In particular, the following will be examined: (a) effect of pretreatment of ESM to reduce the competition for cyanide ion from base metals (mainly copper) present in ESM; (b) effect of pulp densities of ESM on gold recovery; and (c) mutation of the bacteria to grow under alkaline conditions to increase the concentration of cyanide ions available for gold bioleaching.

2. Methods

2.1. Microorganism and growth condition

C. violaceum (ATCC-12472) was purchased from American Type Culture Collection. 1% v/v activated culture was inoculated in 100 ml Luria–Bertani (LB) broth (Miller) and incubated at 30 °C on a rotary shaker at 170 rpm until the bacteria reaches early stationary phase (20–24 h when it reaches maximum cell density and cyanide production). All bacterial stock was supplemented with 30% glycerol and kept in a deep freezer at −80 °C.

2.2. Mutation experiments

Wild *C. violaceum* was exposed to 100 mM of the mutagen, N-Nitroso-N-ethyl urea (ENU) at pH 9, 9.5 and 10 as the selection pressure. The mutations introduced were random and genome wide and may result in a higher chance of targeting the part of the genome that controls pH and growth, although random changes detrimental to other cellular activities may be introduced. However, this method allows the selection of cells capable of growth in alkaline media.

2.3. Electronic scrap

The electronic scrap material used in this study was supplied by Cimelia Resource Recovery Private Limited, Singapore. The grey dust-like ESM (of particle size less than 100 μm) was obtained after shredding and other mechanical separation processes during the recycling of electronic scrap (mainly printed circuit board).

2.4. Pretreatment method

Owing to its abundance in ESM, copper interfered in cyanide-gold ion complex formation and was removed using nitric acid. 10 g of ESM (45–75 μm) was added to 30 ml of 6 M nitric acid in a 250 ml Erlenmeyer flask at room temperature (Mecucci and Scott, 2002) and the mixture was shaken for 2 h. ESM was added incrementally to the nitric acid to prevent excessive frothing due to production of nitric oxide gas. The mixture was centrifuged (5000 rpm for 10 min) and 5 ml of the leachate was extracted for metal analysis using an Inductively Coupled Plasma-Mass Spectrometer (Agilent 7500a ICP-MS). The remaining supernatant was discarded and the residue was washed with deionised water and re-centrifuged. The cycle was repeated until traces of blue-green copper nitrate were no longer visible. The pretreated ESM pellet was finally removed, dried and weighed to constant mass.

2.5. Analytical methods

2.5.1. Acid digestion and metal analysis by ICP-MS

The ESM samples were digested following the protocol of Yamane (Yamane et al., 2011). Samples (1.000 ± 0.005 g) were added in 250 ml Erlenmeyer flasks and digested with 40 ml of aqua regia. The mixture was left to stand for 24 h before centrifugation (5000 rpm, 15 min) and the supernatant sample was kept at 4 °C. The residue was dried and weighed to determine the mass of the metal solubilized. Metal concentrations of acid digested and bioleached samples were analyzed using ICP-MS.

2.5.2. Scanning electron microscopy

Scanning electron microscopy (SEM) analysis was used to observe the morphology of the ESM before and after pretreatment. Dried ESM samples were loaded onto copper stubs using carbon tape and coated with 10 μm platinum particles using an auto fine coater (JEOL JFC-1300) under vacuum at 40 mA for 60 s.

2.5.3. Optical density

Optical densities of bacterial samples were measured using UV–VIS spectrometer (Shimadzu Biospec-mini) at 600 nm. Samples were diluted accordingly to ensure an absorbance within the linear range.

2.5.4. Free cyanide analysis

Free cyanide present in samples was measured using a cyanide electrode (Thermoscientific Orion) connected to Ion Selective Electrode meter. Six point calibration (0, 0.5, 1, 5, 10, 25 ppm) was done weekly.

2.6. Bioleaching studies

Bioleaching of ESM was carried out in 250 ml Erlenmeyer flasks with 100 ml of LB medium at pulp densities of 0.5%, 1%, 2% and 4% w/v. Since direct growth of bacteria and cyanide production are inhibited in the presence of toxic electronic waste in a single-step approach, two-step bioleaching was used in order to obtain higher gold recovery (Brandl et al., 2001; Mishra and Rhee, 2010). In two-step bioleaching, bacteria were initially cultured in LB medium (at 30 °C incubator, 170 rpm) before sterilized ESM was added to the bacterial cultures when the culture has reached significant cell density, and bacterial cyanide production has reached its peak (during early stationary phase). Samples were collected daily, after ESM addition, over eight days. The samples were filtered and analyzed for pH, free cyanide concentration, and heavy metal concentration (in particular, copper and gold).