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RESEARCH ARTICLE | JANUARY 20 2017

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AIP Conf. Proc. 1805, 020004 (2017)
https://doi.org/10.1063/1.4974410

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Bio-Extraction of Precious Metals from Urban Solid Waste

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Abstract. Reduced product lifecycle and increasing demand for electronic devices have resulted in the generation of huge volumes of electronic waste (e-waste). E-wastes contain high concentrations of toxic heavy metals, which have detrimental effects on health and the environment. However, e-wastes also contain significant concentrations of precious metals such as gold, silver and palladium, which can be a major driving force for recycling of urban waste. Cyanogenic bacteria such as *Chromobacterium violaceum* generate cyanide as a secondary metabolite which mobilizes gold into solution via a soluble gold-cyanide complex. However, compared to conventional technology for metal recovery, this approach is not effective, owing largely to the low concentration of lixiviants produced by the bacteria. To overcome the challenges of bioleaching of gold from e-waste, several strategies were adopted to enhance gold recovery rates. These included (i) pretreatment of e-waste to remove competing metal ions, (ii) mutation to adapt the bacteria to high pH environment, (iii) metabolic engineering to produce higher cyanide lixiviant, and (iv) spent medium leaching with adjusted initial pH. Compared to 7.1 % recovery by the wild type bacteria, these strategies achieved gold recoveries of 11.3%, 22.5%, 30% and 30% respectively at 0.5% w/v pulp density respectively. Bioleached gold was finally mineralized and precipitated as gold nanoparticles using the bacterium *Delftia acidovorans*. This study demonstrates the potential for enhancement of biocyanide production and gold recovery from electronic waste through different strategies, and extraction of solid gold from bioleached leachate.

Keywords: e-waste, bioleaching, metabolic engineering, gold recovery, mineralization

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INTRODUCTION

Electronic waste or e-waste is a term used to describe discarded electrical or electronic devices which have reached the end of their useful life. Generally, e-waste is made up of complex mix of metals, plastics and refractory oxides (typically, 40%, 30% and 30%) [1], and contains precious metals such as silver, gold, and platinum, and base metals such as aluminum, copper, nickel, and iron. Globally, e-waste is growing at an alarming rate due to increased consumption of electronic equipment and devices as well as their shorter life span. For instance, the lifespan of central processing units in computers dropped from 4–6 years in 1997 to 2 years in 2005 [2]. Mobile phones have a life span of less than 2 years in developed countries [3]. Indeed, e-waste shows a higher growth rate than any other category of municipal solid waste [3]. United Nations Environment Programme (UNEP) estimates that up to 50 million tons of e-waste is generated globally every year, with only 10% being recycled [4].

The major economic incentive for recycling of e-waste comes from the recovery of commercially valuable metals present in the waste. One ton of electronic scrap from computers, for example, contains more gold than that recovered from 17 tonnes of gold ore, and has 40 times more concentrated copper than that found in copper ore [4]. Thus, the recycling of e-waste not only reduces environmental damage caused by toxic metals (such as lead and cadmium) present in e-waste but also leads to precious metal recovery. Indeed, increased global consumption has also led to pressing need to discover more alternative sources of gold due to depleting natural resources and a worldwide increase in the demand for gold [5].

In the mining industry, naturally-occurring microorganisms have been exploited for extraction of metals from mining waste and lean ores. Microorganisms act as biocatalysts in extracting metals from metal-laden sources [6]. Biomining is an oxidation process, and includes both biooxidation and bioleaching. In biooxidation, microorganisms selectively dissolve undesired minerals from the solid matrix, leaving the metal values of interest enriched in the solid

Proceedings of the 1st International Process Metallurgy Conference (IPMC 2016)
AIP Conf. Proc. 1805, 020004-1–020004-8; doi: 10.1063/1.4974410
Published by AIP Publishing. 978-0-7354-1473-0/$30.00

phase to be recovered by other processes. The resulting supernatant is thereafter discarded. In bioleaching on the other hand, microorganisms (such as bacteria and fungi) convert insoluble solid minerals into soluble form, which can be extracted [7,8]. Here the metal of interest is recovered from the solution. Microorganisms can leach metals through three main principles: (i) redox reactions, (ii) formation of organic or inorganic acids, and (iii) excretion of complexing agents [8]. Currently, industrial extraction of copper, gold, silver, and uranium from their respective ores through bioleaching process is in operation in various parts of the world such as China, Chile, Spain, USA, Australia, Ghana and Brazil [9]. About 20 % of the world's mined copper and 3% of the world's mined gold are generated through bioleaching process [10]. To meet growing demand for metals, solid urban wastes such fly ash, electronic scrap, and spent catalysts may be considered as "secondary ores" for metal recovery. Indeed, with increasing pressure for industry to adopt environmentally friendly and sustainable processes, more attention has been focused on bioleaching as a clean and green technology for metal extraction.

Most bioleaching studies focus on two types of microorganisms, namely mesophilic bacteria and thermophilic archaea. Cyanide is one of the few chemicals that can dissolve gold, a relatively inert element. The reaction between gold and cyanide, known as gold cyanidation, is summarized in Elsner's equation [3]:

$$4Au + 8CN^- + O_2 + 2H_2O \rightarrow 4Au(CN)_2^- + 4OH^- \quad (1)$$

Many cyanogenic bacteria, such as *Chromobacterium violeaceum, Pseudomonas fluorescens, P.aeruginosa, Bacillus megaterium* are capable of producing hydrogen cyanide [11], and form water-soluble metal complexes with metal-containing solids such as printed circuit board scrap [12]. Unlike acidophilic bacteria (such as *Acidithiobacillus thioxidans* and *Acidithiobacillus ferrooxidans*) which are commonly used in bioleaching of heavy metals, cyanogenic microorganisms mobilize metals under alkaline conditions [6].

This work examines the use of *C. violaceum* in gold extraction. In cyanogenic bacteria, cyanide is a secondary metabolite produced in the presence of glycine, a precursor molecule [11]. Cyanide production by *C. violaceum* peaks at the onset of the stationary phase [4], and is produced by oxidative decarboxylation of glycine through catalysis by hydrogen cyanide (HCN) synthase [11] encoded in the hcnABC operon cluster in *C.violaceum* [4]. Regulation of this operon under quorum control restricts its widespread use in metal recovery as the amount of lixiviant produced is low (at about 20 mg/L of cyanide at the onset of stationary phase in a bacterial culture with approximately 1 10^{16} CFU/ml) [4]. To overcome the limited cyanogenic capability of wild-type *C. violaceum*, two strains, pBAD hcn (induced by L-Arabinose) and pTAC hcn (induced by IPTG) were metabolically engineered with an additional copy of the cyanide producing operon (hcnABC) to produce higher cyanide lixiviant. Gold recovery using the two engineered strains and the wild-type *C. violaceum* were investigated and compared to determine if the engineered strains enhanced gold recovery. To overcome the challenges of bioleaching of gold from e-waste, different strategies were also adopted: (i) pretreatment of e-waste to remove competitive copper, (ii) mutation to adapt *C. violaceum* to a high pH environment, (iii) metabolic engineering to produce higher cyanide lixiviant and decoupled from quorum control, and spent medium leaching with adjusted initial pH to enhance gold leaching.

Precious metals from the bioleachate may be recovered using traditional methods such as electrowinning, adsorption onto activated carbon, ion-exchange extraction or zinc cementation process. However, these methods are costly and show low selectivity in multi-ion solution. Bacteria mediated mineralization of gold from the bioleachate can be an environment friendly and sustainable method for its recovery. The bacterium *Delftia acidovorans* produces a secondary metabolite (delftibactin) which mineralizes and precipitates gold from solution [13]. The synthesis of gold nanoparticles (GNPs) using bacteria and without chemical reducing agents renders the process green and sustainable. This is the first study illustrating precious metal recovery from e-waste using different bioleaching strategies for enhanced gold solubilization followed by recovery of gold using gold resistant bacteria *D. acidovorans*.

MATERIALS AND METHODS

Electronic Scrap Material (ESM) and Pre-treatment

Electronic scrap material (ESM) used in this study was provided by Cimelia Resource Recovery Private Limited, Singapore. The metal composition of ESM (of particle size less than 100 μm) has earlier been reported [4]. Owing to its abundance in ESM, copper interferes in cyanide-gold ion complex formation and was removed using nitric acid. Pretreated ESM was used in subsequent leaching experiments.

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Microorganisms 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 ºC on a rotary shaker at 170 rpm until the culture reaches early stationary phase (20–24 hours when it reaches maximum cell density and cyanide production). *D. acidovorans* (ATCC 15668) was grown for 10 hours in modified glycerol peptone broth at 30 ºC in shaker incubator at 150 rpm before use for biomineralization.

Mutation Experiments and Metabolic Engineering

Wild *C. violaceum* was exposed to 100 mM of a 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 growing in alkaline media. *C. violaceum* was metabolically engineered as described earlier [4]. The engineered strains were grown in the presence of the antibiotic gentamycin (15 µg/ml) as selection pressure to prevent contamination from the wild-type strain.

Metal Analysis Of ESM Samples and Free Cyanide Analysis

Samples (1.000 ± 0.005 g) were added in 250 ml Erlenmeyer flasks to 40 ml of aqua regia and stirred for 24 hours. Metal composition of ESM after the acid digestion was determined using an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). Free cyanide in samples was measured using a cyanide electrode (Thermoscientific Orion) connected to Ion Selective Electrode meter.

Bioleaching Experiments

In two-step bioleaching, *C. violaceum* was initially cultured in Luria Bertani, Miller (LB) media in the absence of ESM. Sterilized ESM was added to the media as the second step when the maximum cell density and cyanide production were attained (in the early stationary phase). Two-step bioleaching was adopted to minimize inhibition of cyanide production, owing to toxicity of ESM. In spent medium leaching, cells were separated from the culture after it reached maximum cell density and cyanide production (16–20 hours), and only cell-free metabolites were used for (spent medium) leaching experiments. In all bioleaching experiments, the culture was incubated at 30 ºC and 170 rpm over eight days after addition of ESM.

Biomineralization Experiments

Residual ESM after bioleaching was removed by centrifugation. To the supernatant, *D. acidovorans* was inoculated for biomineralization of the soluble gold complex into gold nanoparticles. Biomineralization experiments was carried out for a period of 10 hours. To obtain Transmission Electron microscopy-electron dispersive X-ray spectroscopic images (TEM-EDX) of gold nanoparticles (GNPs) along with the bacteria, two microliters of bacterial sample were added onto a copper grid. The grid was then washed with 2% phosphotungstic acid and air dried before TEM imaging.

RESULTS AND DISCUSSION

ESM Elemental Composition

The metal composition of the liquor after acid digestion of the original (i.e. untreated) and pretreated ESM are shown in Table 1. The percentage recovery in bioleaching was calculated based on the concentration of each of the metal ions after acid digestion.

Metals made up nearly 30% by mass of ESM; the remaining comprised ceramics, refractory oxides and plastics. Copper constituted the bulk of the untreated ESM, and metals such as silver and gold were present in lower concentration. Base metals such as aluminum, iron, zinc and lead were also present in significant quantities. Copper

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complexes with cyanide, and its presence at a high concentration interferes with gold cyanidation [3]. Other metals such as nickel, iron, silver and zinc also form stable complexes with cyanide [3]. Thus, ESM was pretreated with nitric acid to removed most of the copper (80%) and other metals in order to enhance gold cyanidation in the subsequent bioleaching.

TABLE 1. Elemental composition of untreated and pretreated ESM [3]

Metals	Untreated ESM Composition (mg/g)	Pre-treated ESM Composition (mg/g)	Removed by Pretreatment (%)
Cu	150.40 ± 4.0	30.40 ± 2.1	79.8
Al	47.20 ± 2.8	14.40 ± 1.7	69.5
Fe	31.40 ± 2.0	9.00 ± 0.9	71.3
Pb	28.00 ± 3.2	9.40 ± 1.5	66.4
Sn	17.60 ± 2.2	2.00 ± 0.3	88.6
Ni	16.00 ± 1.8	6.00 ± 0.8	62.5
Zn	11.60 ± 1.2	5.20 ± 0.6	55.2
Ag	0.56 ± 0.09	0.20 ± 0.03	64.3
Au	0.28 ± 0.03	0.24 ± 0.04	14.3
Total	303	76.8	

Figure 1 (a) shows that pretreated ESM yielded higher gold biorecovery compared to untreated ESM (after eight days of two-step bioleaching). The corresponding results for copper recoveries are shown in Fig. 1 (b). In both gold leaching and copper bioleaching, recoveries from the untreated ESM at all pulp densities were lower, compared to that from pretreated ESM at corresponding pulp densities [3]. However, unlike the case of gold leaching, cyanide is not the only lixiviant dissolving copper; the metal could have been leached from ESM in forms other than cyanide-based complex by reagents present in the LB medium. This was confirmed when uninoculated controls also showed copper recovery (Fig 1 (b)). Copper leaching in LB medium has also been reported [6]. As pretreated ESM at 0.5% pulp density resulted higher gold and copper recovery (11.3 and 86.2% respectively) with the wild strain, compared with the original sample (at 7.1% and 71% respectively), it was used to compare the efficiency of gold bioleaching between wild and mutated strains capable of growing under alkaline pH [3].

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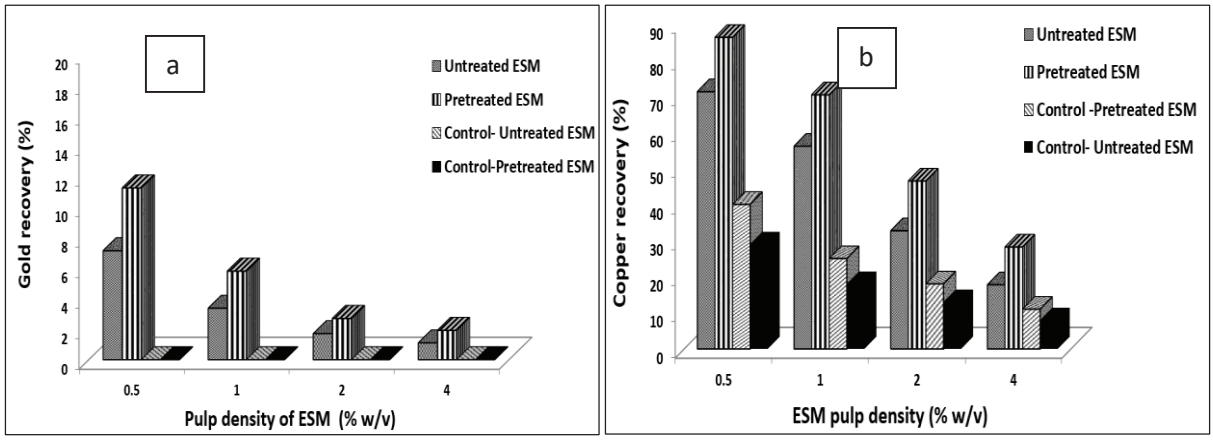

FIGURE 1. Percentage (a) gold and (b) copper recoveries of untreated and pretreated ESM by wild *C. violaceum* at different pulp densities [3]

Two-Step Bioleaching of Gold with Pretreated ESM At 0.5% Pulp Density with Mutated *C. violaceum*

Gold was bioleached from ESM at 0.5% pulp density using the wild and mutated strains (Fig. 2). *C. violaceum* mutated at pH 9.5 gave the highest gold recovery (22.5%), followed by bacteria mutated at pH 10 and pH 9 (at 19% and 18% recovery respectively)[3]. Two factors play an important role in the availability of cyanide for gold bioleaching.

Maximum growth of the bacteria and peak cyanide production occur at initial physiological pH range of 7-8[3]. However, at this pH, cyanide is likely to be lost via volatilization since hydrogen cyanide has a pKa of 9.3; the cyanide concentration remaining in the solution is highly dependent on pH and is favored by a high pH.

Mutation of bacteria to grow under alkaline condition permits growth and cyanide production, and at the same time increases the availability of cyanide ion, thereby improving gold recovery. Although a higher dissociation is expected at pH 10, mutated *C. violaceum* at pH 10 showed lower gold recovery than that at pH 9.5, owing to its significantly lower growth[3]. Wild strain (unadapted) grown at pH 9 and 9.5 recorded higher recovery at 14% and 16% respectively compared to wild strains at pH 7 (11.3%)[3] clearly showing the importance of pH in gold bioleaching. Unadapted strains at pH 10 showed no gold recovery and confirmed that only cyanide produced by the bacteria was responsible for gold bioleaching. Natural adaptation would take a longer time and random mutagenesis may be used to evolve the bacteria more rapidly using pH as the desired selection pressure.

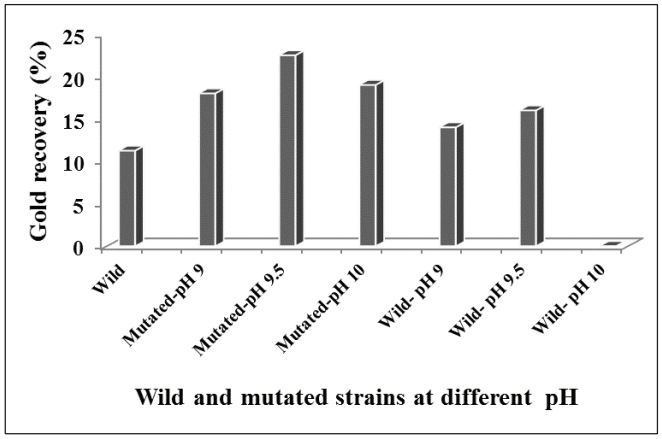

FIGURE 2. Percentage gold recovery of pretreated ESM (0.5% pulp density) in bioleaching by wild and mutated strains at 0.5% pulp density [3]

Gold and Copper Recovery with Engineered Strains

Compared to the wild-type, the engineered strains showed significantly higher gold and copper recovery after 8 days of bioleaching (Fig. 3). At 0.5% w/v pulp density, the engineered strains achieved highest gold recovery of 29.6% (pBAD induced with 0.002% L arabinose) and 24.6% (pTAC induced with 1 mM IPTG) while the wild strain showed a modest recovery of 11.3% (Fig. 3a) [3]. The enhanced gold leaching performance of engineered strains over wild strain is due to increased cyanide production of 34.5 mg/l (pBAD) and 31 mg/l (pTAC) achieved by engineered strains which is significantly higher compared to the wild-type peak concentration of 20 mg/L of cyanide [4]. As pulp density increased, gold recovery decreased for all three strains due to the toxicity of the ESM and the resultant reduction in cyanide production by the bacteria. The corresponding data for copper recovery is shown in Fig. 3 (b) where the pBAD strain again showed highest recovery. However, the difference amongst the 3 strains in copper recovery is not as significant as in gold recovery, possibly due to copper leaching by LB media, in addition to leaching by the biogenic cyanide [4].

Comparison of Gold and Copper Recovery in Two-Step and Spent Medium Leaching

Cyanide concentration in the solution is highly pH dependent [3]. Aqueous CN^- is favoured over gaseous hydrogen cyanide at high pH at equilibrium. At low pH, equilibrium shifts to form more hydrogen cyanide which is volatile and has a low solubility in water. For *C. violaceum,* optimum bacteria growth and peak cyanide production occur at the physiological pH 7-8, here cyanide may be lost via volatilization, since hydrogen cyanide has a pKa of 9.3. Hence, the availability of cyanide ion for gold complexation during two-step bioleaching (at the physiological pH) is not optimal. Apart from bacteria mutation to grow under alkaline condition (as presented earlier), another approach is to decouple bacteria growth and gold complexation, and use the spent medium obtained following peak cyanide

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production under physiological pH. Spent medium leaching at pH 10 (with10 M NaOH addition) resulted in higher gold recovery than that using the mutation approach [3].

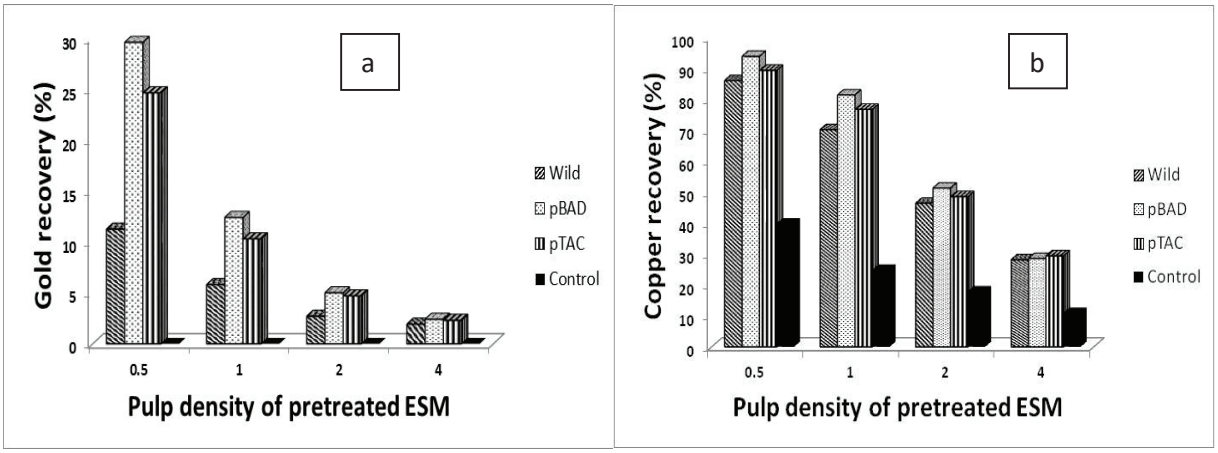

FIGURE 3. Percentage (a) gold and (b) copper recovery of pretreated ESM by wild and engineered strains of *C. violaceum* at different pulp densities [4]

Figure 4 (a, b) compare gold and copper recoveries respectively obtained with two-step bioleaching, spent medium leaching, and spent medium at pH 10, at different pulp densities. Evidently, the highest gold recovery of 30% and copper recovery of 95.7% was obtained with spent medium leaching at pH 10 at 0.5% w/v pulp density [14].

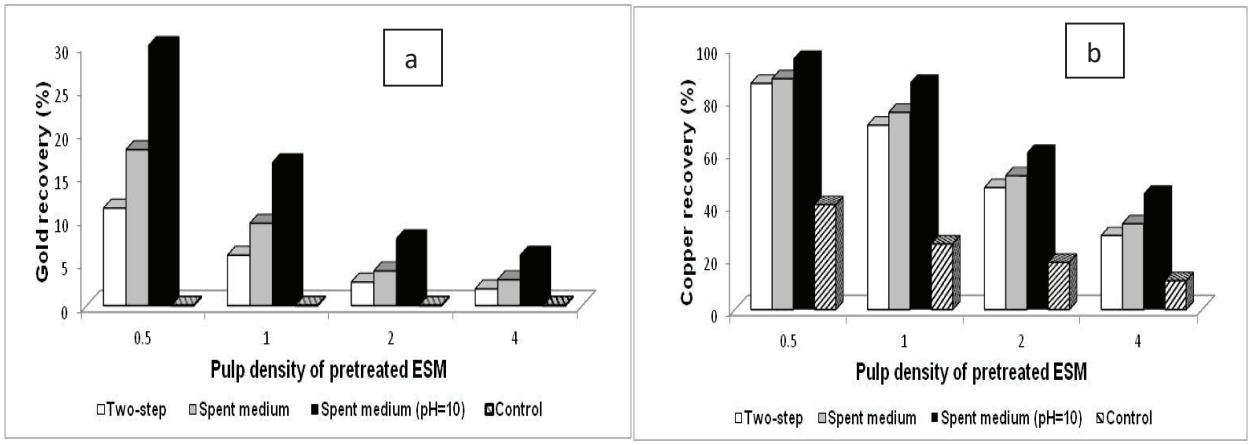

FIGURE 4. (a) Gold recovery and (b) copper recovery from ESM with different leaching methods [14]

Biomineralization Experiments with *D. acidovorans*

D. acidovorans secretes a secondary metabolite delftibactin to protect itself from the toxic nature of Au^{3+} and precipitates gold as nanoparticles in the extracellular medium. 50 ml of the modified glycerol peptone culture medium was added to 50 ml of leachate medium prepared by removing the ESM and bacterial cells after two-step bioleaching. Biomineralization was monitored over 10 hours (Fig. 5(a)). The effect of pH on gold mineralization was examined (Fig. 5(b)); the highest mineralization efficiency was obtained at pH 8 [13].

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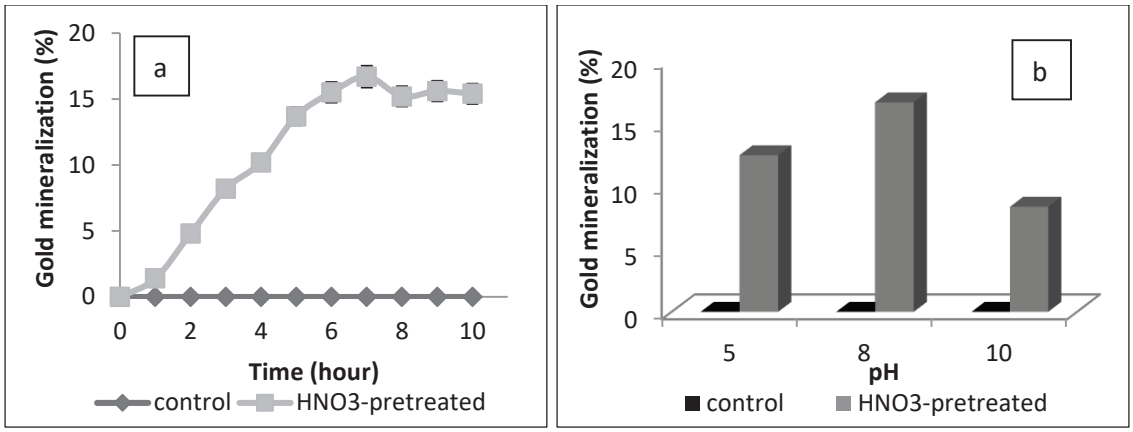

FIGURE 5. (a) Biomineralization profile of gold by *D. acidovorans* in spent medium leachate of 6 M nitric acid pre-treated ESM at pH 8, (b) Effect of pH on biomineralization of gold by *D. acidovorans* [13]

Characterization of Gold Nanoparticles

Transmission electron microscopy-electron dispersive X-ray spectroscopic images (TEM-EDX) of gold nanoparticles (GNPs) along with the bacteria are shown in Fig. 6 (a). GNPs synthesized by *D. acidovorans* were mostly spherical in shape and crystalline in nature, with an average size of 31±2 nm [13]. UV-vis absorption spectra of the GNPs showed an absorption peak at 520 nm which is characteristic of surface plasmon resonance (SPR) band of spherical GNPs at this particle size (Fig. 6 (b)) [13].

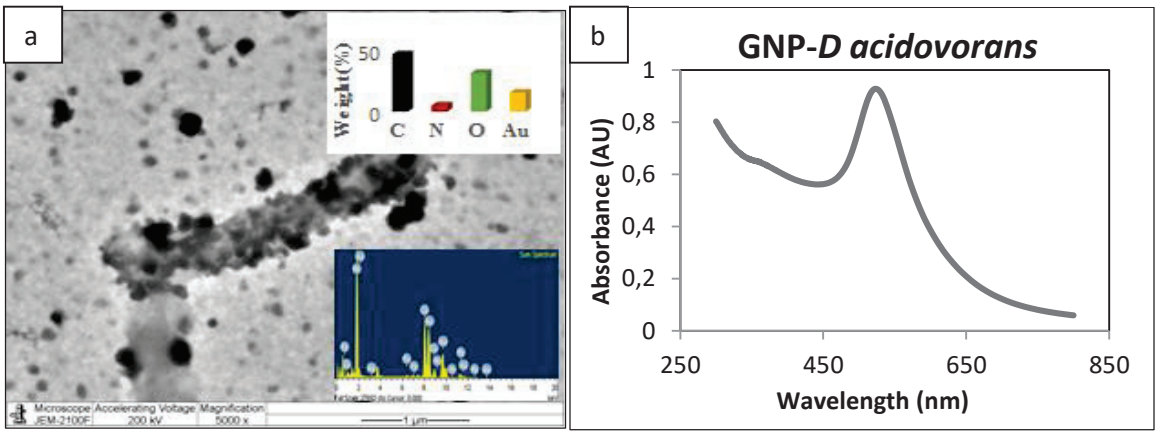

FIGURE 6. (a) TEM-EDX micrographs of GNPs synthesized by *D. acidovorans*; (b) UV–visible absorption spectra of GNPs synthesized by *D. acidovorans*

SUMMARY

This work examined the bioextraction of metals from urban solid waste. Gold was recovered from electronic scrap using a cyanogenic bacterium *C. violaceum*. Nitric acid pretreatment of the waste reduced the competition for cyanide ion from base metals (mainly copper) for metal-cyanide complexation. Three approaches were adopted to increase the concentration of cyanide ions for maximum gold recovery.

In the first approach, mutations were induced in wild *C. violaceum* to enable growth and cyanide production at alkaline pH. Results showed that bioleaching with *C. violaceum* mutated to grow at pH 9.5 (22.5%) resulted in higher gold recovery compared to the unadapted strain (11.3%) and mutated strains at pH 9 (18%) and pH 10 (19%) at 0.5% w/v pulp density. In the second approach, HCN production was increased by using metabolically engineered strains (pBAD and pTAC) for bioleaching with extra copy of cyanide producing operon. Decoupling of cyanogenesis from

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quorum control resulted in a significant increase in cyanide production, and correspondingly, an increase in gold and copper recovery from electronic waste. The pBAD strain produced the highest cyanide concentration, and achieved the highest gold recovery of 30% compared to 11.3% recovery by the wild type bacteria at 0.5% w/v pulp density. In the final approach, performance of spent medium leaching where bacterial growth/cyanide production was decoupled from gold complexation was examined. Spent medium of wild strain gave a higher gold recovery of 18% compared to 11.3% in two-step bioleaching. Addition of alkali to this decoupled process further increased recovery to 30%. Finally, gold was recovered from the leachate through the synthesis of GNPs using *D. acidovorans*.

ACKNOWLEDGEMENTS

This work was support by a grant (R-279-000-354-112) from the Academic Research Fund of the Ministry of Education, Singapore.

REFERENCE

1. E. Y. Sum, JOM **43** (4), 53-61 (1991).
2. B. R. Babu, A. K. Parande and C. A. Basha, Waste Management & Research **25** (4), 307-318 (2007).
3. G. Natarajan and Y.-P. Ting, Bioresource Technology **152**, 80-85 (2014).
4. S. B. Tay, G. Natarajan, M. N. bin Abdul Rahim, H. T. Tan, M. C. M. Chung, Y. P. Ting and W. S. Yew, Scientific Reports **3** (2013).
5. K. M. M. Aung and Y.-P. Ting, Journal of Biotechnology **116** (2), 159-170 (2005).
6. H. Brandl, S. Lehmann, M. A. Faramarzi and D. Martinelli, Hydrometallurgy **94** (1), 14-17 (2008).
7. W. Krebs, C. Brombacher, P. P. Bosshard, R. Bachofen and H. Brandl, FEMS Microbiology Reviews **20** (3-4), 605-617 (1997).
8. D. E. Rawlings, Annual Reviews in Microbiology **56** (1), 65-91 (2002).
9. W. H. Dresher, CWD: INNOVATIONS, May issue, p. 10 (2004).
10. J.-K. Wen, B.-W. Chen, H. Shang and G.-C. Zhang, Rare Metals **35** (6), 433-442 (2016).
11. C. J. Knowles and A. W. Bunch, Advances in Microbial Physiology **27**, 73-111 (1986).
12. M. A. Faramarzi, M. Stagars, E. Pensini, W. Krebs and H. Brandl, Journal of Biotechnology **113** (1), 321-326 (2004).
13. S. Das, B. Dong and Y. P. Ting, Advanced Materials Research **1130**, 668-672 (2015).
14. G. Natarajan and Y.-P. Ting, Chemosphere **136**, 232-238 (2015).

21 May 2025 08:03:43