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Thiourea bioleaching for gold recycling from e-waste

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Abstract

Recycling and reuse of electronic wastes (e-wastes) are becoming an increasingly critical strategy for securing metal resources as well as for minimizing environmental impacts. Thiourea leaching of gold (Au) from e-wastes can be considered an alternative to highly toxic cyanidation, provided that its reagents consumption can be largely reduced. While awareness of the use of biohydrometallurgical techniques in metal mining industries is increasing, the knowledge on microbiological precious metal solubilization is still limited. This led us to investigate and clarify for the first time the potential utility of microbiologically-mediated thiourea leaching (TU-bioleaching) of Au, with a special focus on reducing the reagents consumption while facilitating Au dissolution. Initial screening tests found that different Fe-oxidizing bacteria/archaea possessed varying degrees of thiourea tolerance (5-100 mM). When thiourea and PCB (Printed Circuit Boards) co-exist, *Acidiplasma* sp. Fv-Ap displayed the most robust Fe-oxidation. The Eh level during the reaction was first optimized by fluctuating the initial ratio of thiourea to Fe^{3+} ($TU:Fe^{3+}=2:1-40:1$, by using 1 mM Fe^{3+} vs. 2-40 mM thiourea). The ratio precisely determined the Eh level during the TU-bioleaching and dictated the fate of thiourea decomposition and the resultant Au dissolution from PCB. Microbial contribution to Fe^{3+} regeneration was seen to support steady and continuous Au dissolution, enabling 98% Au dissolution while using low reagent concentrations of 1 mM Fe^{3+} and 10 mM thiourea under the microbial Eh control at around 490-545 mV. This novel TU-bioleaching process offers a new alternative approach for Au recycling from e-wastes and minimization of environmental hazards.

1. Introduction

Production of waste electrical and electronic equipment (e-waste) is one of the fastest growing environmental problems in the world. This is due to the increasing consumption of electronic devices which often end up in landfill sites in developing countries to pollute the surrounding soil and water (Hadi et al., 2015). E-wastes are mixtures of a variety of metals, mainly Cu, Fe and Al, attached to, covered with, or mixed with different types of plastic and ceramics (Guo et al., 2011; Cui and Zhang, 2008). As relatively minor, but high-value components, platinum group metals (i.e., Pd and Pt; used as switching contacts or sensors) and precious metals (i.e., Au and Ag; used as wire-bond in chips, contact surfaces in relays, electroplating layers or conducting paste) can be found in e-wastes (Petter et al., 2014; Teller, 2006). E-wastes can also contain highly toxic metals (i.e., Hg, Cd and As) and organic compounds (i.e., polychlorinated biphenyls and chlorofluorocarbons) (Robinson, 2009) which pose serious concerns to the environment.

As one of the major e-waste producing nations in the world, Japan proposed the 3Rs (Reduce, Reuse, Recycle) initiative at the G8 summit in 2004. Minimization of wastes can be achieved by focusing primarily on "reduce" followed by "reuse" and then "recycle (use of wastes as secondary resources)" (Ministry of the Environment, Japan; www.env.go.jp/recycle/3r/initiative/en/index.html; accessed on 09 Nov 2018). The amount of metal accumulation in Japan to date which is recyclable as a secondary resource (so-called "urban mines") reached a scale comparable to natural reserves in the world's leading resource nations. In fact, Japan's urban mine exceeds 10% of the world reserve of valuable metals such as Au (16%), Ag (22%), In (61%), Sn (11%), and Ta (10%) (National Institute for Materials Science, Japan; www.nims.go.jp/eng/news/press/2008/01/p200801110.html; accessed on 09 Nov 2018). Considering the grade of valuable metals, e-wastes can be regarded as the high-grade surface metal stock, compared to low-grade natural ores. Development of recycling routes for e-wastes is thus gaining increasing importance in order to secure future metal resources as well as to achieve environmental management. Recovery of precious metals such as Au and Ag is a major economic driving force for e-waste recycling.

In the past two decades, the hydrometallurgical process is the most active research area on metal recovery from e-wastes (Cui and Zhang, 2008). Its advantages in the processing of secondary resources are addressed by exactness, predictability and controllability (Andrews et al., 2000). In the precious metal leaching process, the most common leaching agents used include cyanide, thiourea, thiosulfate, halide and ammonia. Among them, thiourea leaching is known to be most effective in acidic conditions (pH 1-2) using Fe^{3+} as an oxidant (Hilson and Monhemius, 2006). While the effectiveness of thiourea leaching has been long proven with natural Au-bearing ores (e.g., Ubaldini et al., 1998), its full-scale operations are still limited, primarily due to the lack of cost-effectiveness compared to conventionally-used cyanide (Cui and Zhang, 2008). The economics of thiourea leaching is basically determined by the reagents consumption and thus its minimization is the most important issue to be solved to improve the process feasibility. Nevertheless, its lower environmental impact together with its fast leaching kinetics makes thiourea still an attractive alternative to highly toxic cyanide (Hilson and Monhemius, 2006).

In thiourea leaching, Au dissolves at acidic pH to form a stable Au-thiourea complex ($AuTU_2^+$) according to Eq. 1:

$$Au + 2CS(NH_2)_2 \rightleftharpoons Au(CS(NH_2)_2)_2^+ + e^- \qquad E^0 = -0.38\ V \qquad [Eq.\ 1]$$

At the same time, thiourea is readily oxidized to formamidine disulfide (FDS) according to Eq. 2:

$$2CS(NH_2)_2 \rightleftharpoons (NH_2)_2CSSC(NH_2)_2^{2+} + 2e^- \qquad E^0 = -0.42\ V \qquad [Eq.\ 2]$$

The above two anode half-reactions are accelerated by strong oxidants such as Fe^{3+}, H_2O_2 and ozone (Marsden and House, 2006), among which Fe^{3+} is most commonly used. The $AuTU_2^+$ complex stability is highly sensitive to pH and Eh (Poisot-Díaz et al., 2008). The rate of thiourea leaching is controlled by the diffusion of reactants, namely, an oxidant, thiourea and FDS. In order to achieve the optimal kinetics, the amount of oxidant was suggested to be set to achieve the ratio of TU:FDS=1:1, but excess oxidative decomposition of thiourea (Eq. 2) must be carefully controlled to improve Au dissolution as well as the cost-effectiveness (Marsden and House 2006; Aylmore, 2016). Further technical developments are essential to address this problem.

So far, several studies employed thiourea leaching for precious metal extraction from e-wastes as well as from natural Au-bearing ores/concentrates, as summarized in Table 1. Biohydrometallurgical technologies (bioleaching and biooxidation) have been successfully used in mining industries, primarily to leach valuable base metals such as Cu from low-grade ores/concentrates (bioleaching) or to remove unwanted mineral sulfides from Au-bearing ores/concentrates as a pre-treatment to leave the metal value in the solid phase (biooxidation). As for solubilization of Au using cyanidation, production of biogenic cyanide by *Chromobacterium violaceum* has been reported to target both natural minerals (Campbell et al., 2001) and e-waste (Li et al., 2015). However, so far to our knowledge, the utility of microorganisms in the thiourea leaching system has never been explained in detail.

Díaz and Roldán (1999) attempted to adapt Fe-oxidizing bacteria to thiourea (10 to 400 ppm), but actual thiourea leaching tests have not been reported. Guo et al. (2017) described the two-step thiourea leaching of Au-bearing concentrates where the attached microbial cells on the washed residue from the first short biooxidation step (6 h) regenerate Fe^{3+} as an oxidant for the second step thiourea leaching. Compared with the 92% Au dissolution in the single-step leaching (without biooxidation pre-treatment), the two-step leaching resulted in 95% Au dissolution. However, due to a minor difference in the Au dissolution between the two processes, the contribution of microbial Fe^{3+} regeneration to the final recovery is unclear and the difference could be the result from the biooxidation pre-treatment (even though it is a short-time process). As the authors also mentioned, more evidence is thus needed to clarify the mechanism.

Based on that, this study attempted to clarify for the first time the effectiveness and mechanism of microbially-mediated thiourea leaching, using e-waste as the target for Au recycling, with a special focus on (i) reduction of chemical reagents consumption and (ii) controlling the Eh level to maximize Au dissolution.

2. Materials and methods

2.1. Characterization of the e-waste sample (PCB)

As the main component of e-waste, the crushed PCB sample, which had undergone magnetic separation, was provided by a recycling company in Japan. The as-received PCB sample was ground with the cryogenic crusher (JFC-2000; Japan Analytical Industry) by adding liquid nitrogen and sieved to collect the particle size of 75-500 μm. The PCB samples before and after grinding and sieving were sputter-coated with Au and observed by scanning electron microscopy (SEM; JSM-7001F, JEOL).

For the elemental analysis, a known amount of the above PCB sample was digested with HNO_3-HCl (2:1, v/v) in the microwave digestion system (Ethos Plus, Milestone) (heated at 1000 W to achieve 230 °C in 30 min, kept at 230 °C for 15 min, and finally allowed to cool to room temperature). The resultant leachate was filtered (0.45 μm membrane), diluted and measured for metal concentrations using inductively coupled plasma-optical emission spectrometry (ICP-OES; PerkinElmer, Optima 8300). The digestion was done in duplicate, and the mean metal contents were calculated.

2.2. Pre-treatment of PCB before thiourea bioleaching

Prior to TU-bioleaching tests, the PCB sample was acid-leached (1 M H_2SO_4 at 70 °C) to extract Cu. After 7-days leaching (97% Cu removed), the solid residue was collected by filtration (0.45 μm), washed thoroughly with distilled water, dried overnight in an oven at 55 °C, and finally divided homogenously by a rotary sample divider (The Fritsch Rotary Cone Sample Divider Laborette 27, Canada).

2.3. Screening of Fe-oxidizing microbes for thiourea tolerance

Prior to the TU-bioleaching tests, several Fe-oxidizing bacterial and archaeal strains (Table 2) were tested for thiourea tolerance. Erlenmeyer flasks (100 mL) containing 50 mL of heterotrophic basal salts (HBS) medium (pH 1.5 with H_2SO_4; per liter; 50 mg KCl, 500 mg $MgSO_4 \cdot 7H_2O$, 50 mg KH_2PO_4, 450 mg $(NH_4)_2SO_4$, 142 mg Na_2SO_4, 14 mg $Ca(NO_3)_2 \cdot 4H_2O$) with respective concentrations of Fe^{2+} and yeast extract were inoculated with each strain at 1.0×10^7 cells/mL, and incubated at respective temperatures (Table 2; shaken at 100 rpm). Thiourea (Wako Pure Chemical Industries) were added to cultures at 0, 5, 25, 50, 100 or 150 mM. Cell densities were monitored by direct microscopic counting using a Thoma counting chamber. Experiments were done in duplicate.

2.4. Thiourea bioleaching

The pre-treated PCB sample (section 2.2) was used for TU-bioleaching.

Acidiplasma sp. Fv-Ap was pre-grown (using the condition in Table 2, plus 20 mM thiourea) and active cells were harvested at the late-exponential phase (by direct microscopic counting using a Thoma counting chamber) by centrifugation (10,000 rpm; 10 min). Cell pellets were washed twice with HBS medium (pH 1.5), prior to inoculation. All TU-bioleaching tests were done in duplicate as follows.

2.4.1. Eh optimization by varying thiourea concentrations

The pre-grown cells were resuspended (at 10^8 cells/ mL) into 100 mL of fresh HBS medium (pH 1.5) in 300 mL flasks containing 3% PCB, 1 mM Fe^{3+} and 0.02 % yeast extract. Thiourea was added to respective flasks at 2, 6, 10, 20 or 40 mM, and incubated at 45 °C, shaken at 150 rpm for 48 h. Cell-free controls were prepared in parallel.

2.4.2. Thiourea bioleaching

The pre-grown cells were resuspended (at 10^8 cells/mL) into 40 mL of fresh HBS medium (pH 1.5) in 100 mL flasks containing 3% PCB, 10 mM thiourea, 1 mM Fe^{3+} and 0.02 % yeast extract. As an anti-decomposition agent for thiourea (Deng et al., 2001), the effect of 0.1 mM Na_2SO_3 addition was also compared. The flasks were incubated at 45 °C, shaken at 150 rpm for 48 h. Cell-free controls were also set up in parallel.

With an attempt to improve Ag recovery, the same experimental set-up was used except that KCl was omitted from the HBS medium and 10, 15 or 20 mM thiourea was tested.

2.4.3. Liquid analysis

Samples were withdrawn periodically to monitor cell density (Thoma counting chamber), pH, redox potential (Eh) and concentrations of Fe^{2+} (*o*-phenanthroline method), total soluble Fe, Au, Ag and Cu (ICP-OES) and free thiourea (Abbasi et al., 2010).

3. Results and discussion

3.1. Characterization of PCB

SEM observation of the as-received PCB sample detected pipe-shaped silicon wafers as major components (Fig. 1a, b), which serve as mechanical support to hold thin metal layers (Olson et al., 2013). Since the as-received PCB sample contained particles and wires of various shapes and hardness, the mechanical pre-treatment method sensitively affected the reproducibility of the leaching results. Using cryogenic crushing was essential in producing homogeneous samples, after which metal layers were liberated from silicon wafers as smaller particles (Fig. 1c, d) and soft Cu wires were also pulverized.

The elemental composition of the PCB sample was determined by acid digestion followed by ICP-OES measurement. Cu was the dominant metal content in the PCB sample (25 ± 0.88% w/w), followed by Fe (1.5 ± 0.08% w/w). Precious metals, Au and Ag were detected at 0.022% ± 0.0002% (0.22 kg/ton) and 0.19% ± 0.03% (1.9 kg/ton), respectively, as the target for TU-bioleaching.

3.2. Screening of the suitable Fe-oxidizing microorganism for TU-bioleaching

In order to select suitable Fe-oxidizer(s) for the following TU-bioleaching, several strains were screened for thiourea tolerance. Microbial growth was correlated with the degree of Fe^{2+} oxidation. Growth of the mesophilic *Fm. acidiphilum* T23 (Fig. 2a) and *Fp. acidiphilum* Y (Fig.

2c) was mostly unaffected by 5 mM thiourea, but severely inhibited by ≥25 mM thiourea. The moderately thermophilic *Sb. acidophilus* YTF1 was much more resistant to thiourea and a major delay in growth was first observed at 100 mM thiourea (Fig. 2b). Addition of 5 mM thiourea delayed the growth of moderately thermophilic *Acidiplasma* sp. Fv-Ap, and 25 mM thiourea allowed its partial growth (Fig. 2d). As for thermophilic archaea (*Ac. brierleyi* and *S. tokodaii* 7), 5 mM thiourea showed relatively minor effect (Fig. 2e, f) and growth of the latter recovered after while even at 25-50 mM thiourea (Fig. 2f). However, given the observation that decomposition of thiourea becomes more significant at elevated temperatures (Wang et al., 2005), thiourea tolerance of the two thermophiles may have been overestimated (although it is possible that the decomposition products also possess some inhibitory effect). It can be also speculated that the decomposition products serve as nitrogen sources for heterotrophic strains at non-inhibitory concentrations. The most thiourea-tolerant *Sb. acidophilus* YTF1 is known to be a "weak" (relatively slower) Fe-oxidizing moderate thermophile, whilst its archaeal counterpart *Acidiplasma* sp. Fv-Ap was less thiourea-tolerant but has a "stronger" (relatively faster) Fe-oxidizing ability (Masaki et al., 2018). Therefore, as the next step, the two moderate thermophiles were compared for their Fe-oxidizing activity in the presence of 10 mM thiourea plus 3% PCB (data not shown): Unexpectedly, the former was unable to regenerate Fe^{3+} probably due to its sensitivity to the PCB, whereas the latter was able to do so. Based on the overall results, the following TU-bioleaching tests were conducted using *Acidiplasma* sp. Fv-Ap.

3.3. Effect of the Eh level on Au dissolution

The initial molar ratio of thiourea to Fe^{3+} (TU:Fe^{3+}) was set to 2:1, 6:1, 10:1, 20:1 or 40:1, by use of 1 mM Fe^{3+} plus 2, 6, 10, 20 or 40 mM thiourea, respectively, to find the optimal condition for Au recovery (Fig. 3). As a general trend regardless of the presence of cells, use of higher thiourea concentrations resulted in lower Eh values during the leaching reaction. The initial Eh level of around 800 mV (at 0 min) dropped at the moment of PCB addition (Fig. 3a, c). The average Eh values (Eh_{ave}) calculated between 0.25-48 h after addition of PCB were 550, 520, 457, 419 and 422 mV in cell-free controls (Fig. 3a) and 580, 521, 504, 458 and 429 mV in the presence of *Acidiplasma* sp. Fv-Ap cells (Fig. 3c) at TU:Fe^{3+} = 2:1, 6:1, 10:1, 20:1 and 40:1, respectively.

Abiotic system:

Upon addition of PCB, the majority of Fe^{3+} ions were rapidly reduced to Fe^{2+} during the first hour in all cases (based on Eq. 3 and Eq. 4; Marsden and House 2006). However, the amount of persisting soluble Fe^{3+} was more noticeable when lower initial thiourea concentrations were used (data not shown), consequently keeping higher Eh levels (Fig. 3a). Because of the balance between Eq. 3 and Eq. 4, elevating the initial thiourea concentration did not necessarily improve Au dissolution (Fig. 3b). An initial delay in Au dissolution at higher thiourea concentrations (20 mM and 40 mM) implies that consumption of Fe^{3+} was partly directed towards oxidation of thiourea to FDS (Eq. 4), instead of towards formation of $AuTU_2^+$ (Eq. 3). Still, a small amount of Fe^{3+} was regenerated by chemical oxidation (as can be seen from a slight increase in the Eh level; Fig. 3a) which supported slow but continuous Au dissolution at the later stage (Fig. 3b). Consequently, final Au dissolution was slightly lower (55% or 56%) at 20 mM or 40 mM thiourea, respectively, compared to those at 6-10 mM thiourea (Fig. 3b). Use of the lowest thiourea concentration (2 mM) triggered decomposition of $AuTU_2^+$, especially after 8 h, to result in only 16% final Au dissolution. This is due to the high Eh condition promoting irreversible disproportionation of FDS (Eqs. 5-6; Marsden and House 2006) as well as destabilization of the $AuTU_2^+$ complex (Poisot-Díaz et al., 2008). Formation of S^0 (Eq. 5) and H_2S (Eq. 6) is undesirable due to surface passivation and reprecipitation of Au (Marsden and House 2006). In the abiotic system, use of 6-10 mM thiourea led to the highest Au dissolution of 60-64% (Fig. 3b).

$2Au^0 + 4CS(NH_2)_2 + 2Fe^{3+} \rightleftharpoons 2Au(CS(NH_2)_2)_2^+ + 2Fe^{2+}$ [Eq. 3]

$2CS(NH_2)_2 + 2Fe^{3+} \rightleftharpoons NH_2(NH)CSSC(NH)NH_2 + 2Fe^{2+} + 2H^+$ [Eq. 4]

$NH_2(NH)CSSC(NH)NH_2 \rightleftharpoons CS(NH_2)_2 + S^0 + CN(NH_2)$ [Eq. 5]

$CS(NH_2)_2 + H_2O \rightleftharpoons CO(NH_2)_2 + H_2S$ [Eq. 6]

Biotic system:

Here, the Eh_{ave} levels were generally higher than in cell-free controls at the same thiourea concentrations. The Eh values also gradually increased during the leaching reaction (Fig. 3c). This clearly indicates that *Acidiplasma* sp. Fv-Ap played a role to re-generate Fe^{3+} (Eq. 7) to support continuous Au dissolution.

$4Fe^{2+} + O_2 + 4H^+ \rightleftharpoons 4Fe^{3+} + 2H_2O$ [Eq. 7]

At lower thiourea concentrations (2 mM or 6 mM), microbial Fe^{2+} oxidation negatively or neutrally affected the final Au dissolution (0.4% or 62%, respectively; Fig. 3d) compared to the respective cell-free control (16% or 60%, respectively; Fig. 3b). This was again due to the resultant higher Eh levels (Fig. 3c) that tend to facilitate decomposition of thiourea (Eqs. 4-6) and destabilization of the $AuTU_2^+$ complex (Poisot-Díaz et al., 2008). At higher thiourea concentrations (20 mM or 40 mM), some positive microbial effect on Au dissolution was seen (61% or 63%, respectively; Fig. 3d), compared to the respective cell-free control (55% or 56%, respectively; Fig. 3b). This effect of microbial Fe^{3+} regeneration became most noticeable at 10 mM thiourea, achieving 98% Au dissolution (Fig. 3b); wherein, the Eh value increased from 490 mV (0.25 h) to 545 mV (48 h) with Eh_{ave} of 504 mV, to continuously facilitate the formation of $AuTU_2^+$ (Fig. 3d). Comparison of the overtime change in Eh and Au dissolution at 6 mM and 10 mM thiourea (Fig. 3c, d) suggests that in order to support steady and continuous Au dissolution, it is important to maintain the Eh level below 500 mV for initial hours while simultaneously regenerating Fe^{3+}.

3.4. Thiourea bioleaching

Based on the findings in section 3.3, TU-bioleaching tests were conducted to follow the leaching behavior of major metals of interest, under the optimal condition (10 mM thiourea and 1 mM Fe^{3+}) (Fig. 4). Among those various additives tested so far in literature (Aylmore 2016), this study evaluated the effect of Na_2SO_3 (Deng et al., 2001) as an anti-decomposition agent for thiourea, by reversing the oxidation reaction of thiourea (Eq. 4) before FDS is irreversibly disproportionated (Eqs. 5-6), according to Eq. 8 (Guo et al., 2017).

$$NH_2(NH)CSSC(NH)NH_2 + SO_3^{2-} + H_2O \rightleftharpoons 2CS(NH_2)_2 + SO_4^{2-} \qquad [Eq. 8]$$

Regardless of the presence of Na_2SO_3, the dissolution behavior of Au and Ag (Fig. 4a, b) and the trend in microbial Fe oxidation and the resultant Eh values (Fig. 4 c, d) were almost identical. The pH values were unchanged throughout the reaction (data not shown). In the biotic system without Na_2SO_3 addition, 98% Au (Fig. 4a) and 14% Ag (Fig. 4a) were dissolved. From the residual Cu^0 remained after the pre-treatment, 55% Cu (0.09 mM Cu) was dissolved (data not shown). Thiourea was reported to form strong complexes not only with precious metals but also with some base metals such as Cu (Aylmore, 2016; Marsden and House, 2006). Stoichiometrically, in the absence of Na_2SO_3, a total of 0.6 mM thiourea was complexed with metals (0.08 mM $AuTU_2^+$, 0.12 mM $AgTU_3^+$ and 0.4 mM $CuTU_4^+$), while 7.8 mM thiourea was oxidized to FDS, leaving 1.6 mM intact thiourea in the solution at 48 h (Fig. 4e). On the other hand, in the presence of 0.1 mM Na_2SO_3, a total of 0.6 mM thiourea complexed with the metals, while 3.0 mM thiourea was oxidized to FDS to leave 6.4 mM intact thiourea in the solution (Fig. 4e). Therefore, the addition of Na_2SO_3 to the biotic system prevented thiourea decomposition by 62% (Fig. 4e). The effectiveness of the Na_2SO_3 addition was also clear from Fig. 4f, shifting the TU:FDS ratio even at the end of the leaching reaction (48 h) from 1:5 to 2:1 in the biotic system.

In contrast to the trend of Au, dissolution of Ag had mostly ceased after 6 h in all cases (Fig. 4b). Dissolved Ag (as $AgTU_3^+$) even started to rapidly decompose in the biotic system, likely due to the increased Eh level which was less suitable for stabilization of $AgTU_3^+$ complexes (Poisot-Díaz et al., 2008). Removing KCl salts from the bioleaching medium allowed stabilization of $AgTU_3^+$ significantly, by preventing precipitation of AgCl (Fig. 5b). Unlike the case of Au, use of 20 mM TU, instead of 10 mM TU, was optimal to maximize the final Ag recovery (> 80%; Fig. 5b), due to the lower Eh level established under this condition (Fig. 5c). Even at the same Eh level, Au dissolution was negatively affected by the absence of KCl: i.e., The final Au recovery was 98% or 75% at 10 mM TU with or without KCl. This might be due to the chloride leaching effect of Au (Baghalha, 2007).

As for Cu, even after the pre-treatment of PCB, the residual Cu readily dissolved in all cases almost to the same extent. This competed with the dissolution of precious metals, since $CuTU_4^+$ complexes are stable in the wider Eh range, compared to the case of Au and Ag (Poisot-Díaz et al., 2008).

Marsden and House (2006) mentioned that thiourea concentrations of 5-50 g/L (65-650 mM) had been applied for laboratory- and pilot-scale tests. As summarized in Table 1, roughly 100-1000 mM thiourea have been typically used in previous studies together with up to ~100 mM Fe^{3+} as an oxidant in the pH range 0-1.7. When calculated based on the Au content in each sample, the amount of thiourea and Fe^{3+} used (Au:TU:Fe^{3+} in Table 1) were generally much greater than that used in this study. The results in this study indicated that the reagents consumption for thiourea leaching could be largely reduced by incorporating microbial activity (for Fe^{3+} regeneration and Eh control) while realizing nearly complete Au dissolution. The study provides yet fundamental results but provides evidence to propose a new technical approach for precious metal bioleaching. Although this novel TU-bioleaching process would need a longer reaction time (than conventional reagent-consuming chemical thiourea leaching) to show its best performance, the mechanism can potentially be applied to develop alternative low-cost technologies for precious metal bioleaching targeting both e-wastes and natural Au-bearing ores (e.g., solution mining and heap biooxidation coupled with TU-bioleaching). Previously, Wan et al. (1995) reported a pilot heap thiourea leaching test following the biooxidation pre-treatment, using 10 g/L (130 mM) thiourea solution pumped onto the heap at $pH < 2.5$ and Eh at 0.43-0.5 V (vs. SHE) (without chemical adjustment of pH and Eh). The overall result was not satisfactory, and some physicochemical factors such as cold temperature and large particle size were seen as a problem. Recirculation of the solution led to the formation of S^0 passivation onto activated carbons and resins, impeding the Au absorption process. Involvement of microbial activity in this heap thiourea leaching study is unknown, but condition optimization in order to drive microbial Fe-oxidation (for Eh control) and S-oxidation (removal of passivation) may enable improvement in Au recovery. In the case of e-waste leaching, a combination of base metal bioleaching followed by TU-bioleaching of precious metals could become a potential sequential biohydrometallurgical approach that takes the full advantage of microbial reactions. Another advantage is that pre-oxidized sulfide ores or e-wastes can be directly processed in the next thiourea leaching step without a neutralization treatment.

3. Conclusions

- Different Fe-oxidizing bacteria/archaea exhibited a different degree of thiourea tolerance (5-100 mM).
- Among those tested, *Acidiplasma* sp. Fv-Ap shared both thiourea and PCB tolerance to display the most robust Fe-oxidation in the leaching reaction.
- The initial TU:Fe^{3+} ratio (1 mM Fe^{3+} vs. 2-40 mM thiourea) precisely determined the Eh level during the leaching reaction and dictated the fate of thiourea decomposition and the resultant Au dissolution from PCB.
- The greatest Au dissolution of 98% was achieved using low reagent concentrations (1 mM Fe^{3+} and 10 mM thiourea) via microbial Fe^{3+} regeneration and under the simultaneous Eh control at 490-545 mV.
- Compared to the past studies, reduction of the reagents consumption was shown possible, while facilitating Au dissolution.
- As the highlight of the study, the effectiveness of the novel TU-bioleaching was demonstrated for the first time. This mechanism can be potentially applied to develop alternative technologies for precious metal bioleaching of e-wastes. The possibility to use the TU-bioleaching process without a neutralization step after biooxidation of natural gold-bearing ores was also implied.

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Figure legends

Fig. 1. SEM images of the PCB sample before (a, b) and after (c, d) grinding and sieving (-500+75 μm).

Fig. 2. Inhibitory effect of thiourea on the growth of different Fe-oxidizing bacteria/archaea: (a) *Fm. acidiphilum* T23, (b) *Sb. acidophilus* YTF1, (c) *Fp. acidiphilum* Y, (d) *Acidiplasma* sp. Fv-Ap, (e) *Ac. brierleyi*, (f) *S. tokodaii* 7. Thiourea concentrations tested were 0 mM (○), 5 mM (■), 25 mM (▲), 50 mM (▼), 100 mM (◀) and 150 mM (◆). Data points are mean values from duplicate experiments.

Fig. 3. Correlation between the Eh level (a, b) and Au dissolution (c, d) in abiotic cultures (cell-free controls; a, c) and biotic cultures (bioleaching with *Acidiplasma* sp. Fv-Ap cells; b, d). The molar ratio of thiourea to Fe^{3+} (TU:Fe^{3+}) was set to 2:1, 6:1, 10:1, 20:1 or 40:1, by use of 1 mM Fe^{3+} plus 2 mM (▲,△), 6 mM (*,×), 10 mM (■, □), 20 mM (●,○) or 40 mM (◆,◇) of thiourea, respectively. Data points are mean values from duplicate cultures.

Fig. 4. Changes in the (a) total Au concentration, (b) total Ag concentration, (c) Fe^{2+} (broken lines) and total Fe (solid lines) concentrations, (d) Eh value, (e) free thiourea concentration and (g) TU/FDS ratio in bioleaching cultures with *Acidiplasma* sp. Fv-Ap cells (▲, ●) and cell-free control cultures (△, ○) with (●, ○) or without (▲, △) addition of 0.1 mM Na_2SO_3. Data points are mean values from duplicate cultures.

Table legends

Table 1

Summary of thiourea leaching studies.

Table 2

Growth conditions used in the screening of Fe-oxidizing bacteria/archaea for thiourea-tolerance.