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Effective and selective recovery of gold and palladium ions from metal wastewater using a sulfothermophilic red alga, *Galdieria sulphuraria*

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HIGHLIGHTS

- *Galdieria sulphuraria* cells absorbed precious metals from HCl solution.
- They selectively recovered Au^{3+} and Pd^{2+} from aqua regia-based metal wastewater.
- Au^{3+} and Pd^{2+} can be eluted from *G. sulphuraria* as soluble complexes.
- The selective recovery and elution process can be completed within 1 h.

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ABSTRACT

The demand for precious metals has increased in recent years. However, low concentrations of precious metals dissolved in wastewater are yet to be recovered because of high operation costs and technical problems. The unicellular red alga, *Galdieria sulphuraria*, efficiently absorbs precious metals through biosorption. In this study, over 90% of gold and palladium could be selectively recovered from aqua regia-based metal wastewater by using *G. sulphuraria*. These metals were eluted from the cells into ammonium solutions containing 0.2 M ammonium salts without other contaminating metals. The use of *G. sulphuraria* is an eco-friendly and cost-effective way of recovering low concentrations of gold and palladium discarded in metal wastewater.

1. Introduction

Gold and platinum belong to the "precious metals" group, and their demand for applications in the fields of medicine, electronics, and catalysis has been increasing in recent years. Because of their importance, various recycling methods (e.g. solvent extraction, ion exchange chromatography, and pyrometallurgical processes) have been developed (Das, 2010). However, such processes are costly and generate large quantities of secondary wastes. In addition, practical metal wastewater contains precious metals at concentrations below 10–40 mg/L, which cannot be recycled currently (Umali et al., 2006; Umeda et al., 2011). Biological methods have been proposed as an eco-friendly and cost-effective approach to recover precious metal ions from aqueous solutions (Das, 2010), and the kinetics and mechanisms that may be employed for the recovery of precious metals have been studied in many microorganisms (Mack et al., 2007; Won et al., 2014). Nevertheless, biological methods have not been applied in practical recycling processes. One of the reasons is that metal wastewater contains very low concentrations of precious metals, while it contains strong acids and high concentrations of base metals such as iron and copper. Therefore, more effective and selective recovery methods under acidic conditions need to be optimized for the practical use of biological methods in precious metal recovery.

Galdieria is a unicellular microalga belonging to the Cyanidiales family of red algae. *Galdieria* is a predominant species in hot sulfur springs (pH < 5; temperature, up to 56 °C), and it is adapted to highly acidic conditions (Fukuda, 1958; Ascione et al., 1966).

Abbreviations: ICP-MS, inductively coupled plasma-mass spectrometry; SEM–EDS, scanning electron microscopy and energy dispersive X-ray spectrometry.

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Because Cyanidiales adapt to high-temperature, acidic, metal-rich environments, they possess unique metal-tolerant mechanisms (Yoshimura et al., 2000; Nagasaka et al., 2003; Minoda et al., 2015). Among the Cyanidiales, *Galdieria* is a suitable alga for use in biotechnology because it can tolerate various environmental stresses and produce large amounts of biomass and beneficial compounds (Gross and Schnarrenberger, 1995; Graverholt and Eriksen, 2007; Selavaratnam et al., 2014). In this study, we investigated the efficient and selective adsorption of low concentrations of gold and palladium from aqua regia-based metal wastewater by *Galdieria sulphuraria*. Additionally, we recovered gold and palladium ions of high purity from the cells.

2. Methods

2.1. Algal culture

Five-hundred milliliters of autotrophic *G. sulphuraria* 074 W cells were grown in Modified Allen's Medium in glass vessels as described previously with minor modifications (Allen, 1959; Gross and Schnarrenberger, 1995; Minoda et al., 2004). Cells were grown in 2 × Allen's Medium containing the following salts (g/L): $(NH_4)_2SO_4$, 2.62; KH_2PO_4, 0.54; $MgSO_4{\cdot}7H_2O$, 0.5; $CaCl_2{\cdot}2H_2O$, 0.14; and $FeCl_3{\cdot}6H_2O$, 0.016. The following trace elements were included at double the concentration (g/L) of Arnon's A_6 solution without H_2BO_3: $MnCl_2{\cdot}4H_2O$, 1.8; $ZnCl_2$, 0.105; $Na_2MoO_2{\cdot}2H_2O$, 0.39; $CoCl{\cdot}6H_2O$, 0.04; and $CuCl_2$ 0.043. Cells were incubated at 42 °C under light (70 μE/m^2 s).

2.2. Metal recovery and elution

For the metal recovery experiments, cells were harvested at the middle-logarithmic phase (OD_{750} = 1) followed by washing with 0.4 M hydrochloric acid solution. For the recovery experiments, cells were incubated with each metal solution for 30 min (concentrations are indicated in the respective figure legends). All metals were added as chloride salts at the concentrations indicated in the figure legends. For the elution experiments, cells were incubated with metal wastewater, which was diluted ten times, for 15 min and collected by centrifugation. After being washed with H_2O, the cells were suspended in each elution solution and incubated for 30 min. The concentration of the metals in the supernatant of the solution was determined by inductively coupled plasma-mass spectrometry (ICP-MS, ELAN DRC-e, Perkin Elmer, US).

2.3. Measurement of metal concentrations by ICP-MS

After 30 min of incubation, the cell suspension was separated into supernatant fractions and cell fractions by centrifugation at 4000*g* for 5 min. After centrifugation, the metal concentration of the supernatant was determined by ICP-MS with Te as an internal standard. A solution without cells, labeled as '−Cell', was treated in the same way as the cell suspension, and the metal concentration of the supernatant was determined. Removal efficiencies were calculated by dividing the metal concentrations of the supernatants by those of the '−Cell' samples.

2.4. Elemental analysis of single cells by time-resolved ICP-MS

Using our previously developed ICP-MS system (Miyashita et al., 2014), the prepared *G. sulphuraria* cell suspensions, with or without incubation with metal ions, were directly introduced into the plasma of the ICP-MS system as an aerosol through the modified high efficiency cell introduction system for time-resolved ICP-MS measurements at an integration time per data point of 1 ms. Spike signals corresponding to cell events were detected for P and some other elements upon introduction of the cell suspension. Since a single spike of P is derived from a single cell, P spike is used as index of cell and the number of P spikes is corresponding to cell number (Miyashita et al., 2014). Cells were diluted 100 times with 0.4 M hydrochloric acid, 10 times with 40 mM hydrochloric acid, or 500 times with diluted aqua regia for direct injection into the ICP-MS system.

3. Results and discussion

3.1. High efficiency recovery of precious metals (Au^{3+}, Pd^{2+}, and Pt^{4+}) using G. sulphuraria cells through biosorption

Since low concentration of precious metals cannot be recycled currently (Umeda et al., 2011), the recovery of precious metals (Au^{3+}, Pd^{2+}, and Pt^{4+}) under 25 mg/L were tested using *G. sulphuraria* cells (Fig. 1). Cells were incubated with precious metals dissolved in hydrochloric acid solution for 30 min. In 40 mM hydrochloric acid solution (pH 2.5), over 80% of Au^{3+} and Pd^{2+} were removed from the solution using cells at 1.4 mg/mL (dry weight) in the range of 0.5–25 mg/L (Fig. 1a and b), while cells at 14 mg/mL were required for the recovery of over 60% of Pt^{4+} (Fig. 1c). In 0.4 M hydrochloric acid solution (pH 0.5), the removal efficiency of Pd^{2+} decreased as increase in the concentration of Pd^{2+}, finally reaching 20% at 25 mg/L. Pt^{4+} ions were not removed from the 0.4 M hydrochloric acid solution (pH 0.5) entirely. On the other hand, although the removal efficiency of Au^{3+} decreased to 50% at 0.5 and 25 mg/L in 0.4 M hydrochloric acid solution (pH 0.5), it was still higher than the efficiencies obtained for Pd^{2+} and Pt^{4+}. Precious metals were not removed from hydrochloric solutions after 30 min of incubation without cells (Table S1). Scanning electron microscopy and energy dispersive X-ray spectrometry (SEM–EDS) analysis confirmed the presence of precious metals on the cell surface (Fig. S1 and Tables S2 and S3), but the signal was very weak because of the low concentration of added precious metals and the influence of glutaraldehyde used in the sample preparation for SEM.

To detect the presence of Au^{3+}, Pd^{2+}, and Pt^{4+} in the cells, we used time-resolved inductively coupled plasma mass spectrometry. This method enables the detection of trace concentrations of metals contained in a single cell via direct introduction of a single cell into the ICP-MS system (Miyashita et al., 2014). Thus, no pretreatment is required. The signal height for phosphate, which is derived from the cells, increased in the cell fractions, compared with the fractions without cells (Fig. 2). The signal frequencies of Au^{3+}, Pd^{2+}, and Pt^{4+} increased only in the cell fractions, suggesting that these precious metals were accumulated in the cells. Reproducible results were obtained from three biological replicates (Figs. 2 and S5). Single phosphate signal is derived from a single cell and frequency of phosphate signals is corresponding to cell number. In the Au^{3+} or Pd^{2+} samples, the signal frequencies of the precious metals were lower than that of phosphate, while the signal heights of the precious metals were significantly higher than that of phosphate (Fig. 2a and b). This suggests that the precious metals were accumulated in the small part of the cells with high concentration. The frequencies of the Pt^{4+} signals were higher than those of the Au^{3+} or Pd^{2+} signals, but this was because the cell concentration of the Pt^{4+} sample was 10-times higher than those of the Au^{3+} and Pd^{2+} samples to compensate for the 10-fold lower concentration of added Pt^{4+} (Fig. 2c). In the case of Pt^{4+} samples, the signal heights for phosphate were several times higher than those for Au^{3+} and Pd^{2+}. It is unlikely that phosphate accumulation was stimulated by the addition of Pt^{4+} during the 30 min of incubation in hydrochloric acid solution; therefore, this increase in signal

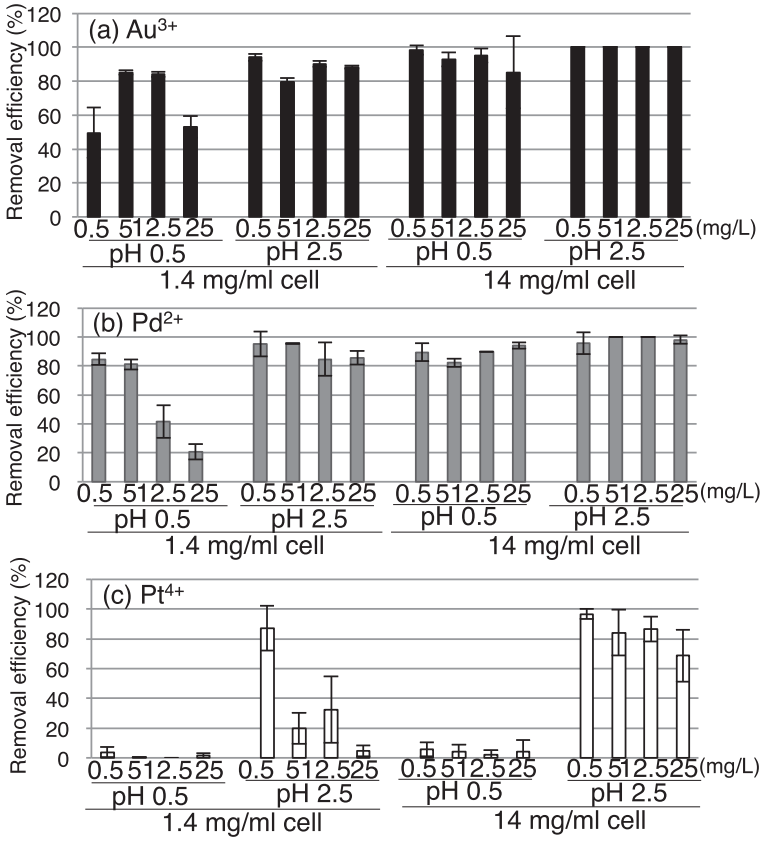

Fig. 1. Removal efficiencies of Au^{3+}, Pd^{2+}, and Pt^{4+} from hydrochloric acid solution using *Galdieria sulphuraria*. Cells were incubated for 30 min in 0.4 M hydrochloric acid solution (pH 0.5) or 40 mM hydrochloric acid solution (pH 2.5) containing Au^{3+}, Pd^{2+}, or Pt^{4+} at the concentrations indicated in the figure. Cells corresponding to 1.4 mg/mL or 14 mg/mL (dry weight) were added into the solutions. Removal efficiency was determined by dividing the soluble metal concentration of each fraction by soluble metal concentration after 30 min of incubation without cells (Table S1). Values are expressed as average ± standard deviation (SD) of three independent experiments.

height was probably caused by cell aggregation. Thus, the precious metals were accumulated in the *G. sulphuraria* cells at high concentrations within 30 min of incubation.

The recovery efficiencies of the precious metals in hydrochloric acid solution were almost the same as those in *Chlorella vulgaris*, *Escherichia coli*, and *Pseudomonas maltophilia*, which were reported as microorganisms that could recover precious metals at concentrations below 100 mg/L with high efficiency (Greene et al., 1986; Nakajima, 2003; Godlewska-Żyłjuewucz, 2003) at pH 2. The decrease in the recovery efficiency at pH 0.5 was also consistent with the results reported by studies involving *C. vulgaris*, *P. maltophilia*, and *Saccharomyces cerevisiae* (Greene et al., 1986; Nakajima, 2003; Godlewska-Żyłjuewucz, 2003; Żyłjuewucz and Kozłowska, 2005). In these organisms, highly efficient recovery of precious metals occurred through biosorption on the cell surface between the chloride complexes of the precious metals and the positive charges on the cell surface (mainly positively charged amine residues) under acidic conditions (Greene et al., 1986; Wang et al., 2015). In our study, there was no difference in the recovery efficiency of precious metals between living and freeze–thawed *G. sulphuraria* cells at 40 °C, indicating that the recovery of precious metals by *G. sulphuraria* was caused by biosorption, in the absence of cell metabolism (Fig. S2). SEM observations also showed that the precious metals likely did not form nanoparticles or salts on the cell surface in 30 min (Fig. S1). On the other hand, the removal efficiencies of Pd^{2+} and Pt^{4+} decreased at 4 °C (Fig. S2). Biosorption is usually not dependent on temperature, but in the case of Pd^{2+} and Pt^{4+}, the recovery efficiency is affected by temperature because of endothermal reactions (Wang et al., 2015). This might explain the decrease in the removal efficiencies of Pd^{2+} and Pt^{4+} in *G. sulphuraria* at 4 °C.

3.2. Selective recovery of precious metals (Au^{3+} and Pd^{2+}) from aqua regia-based metal wastewater using G. sulphuraria cells

Wastewater containing precious metals usually exists in the form of a solution of cyanide or aqua regia (mixture of hydrochloric acid and nitric acid at a ratio of 3:1). We tested the recovery of precious metals using *G. sulphuraria* from wastewater, which was diluted with aqua regia containing 5.6 M acid, 570 mg/L $Fe^{2+/3+}$, 4800 mg/L Cu^{2+}, 40 mg/L Pt^{4+}, 530 mg/L Au^{3+}, 460 mg/L Ni^{2+}, 50 mg/L Sn^{2+}, 120 mg/L Pd^{2+}, and 110 mg/L Zn^{2+}. Without any dilution, no metals were removed from the metal wastewater using *G. sulphuraria* cells after 30 min of incubation (Table S4). However, after 30 min of incubation, over 90% of Au^{3+} and Pd^{2+} were effectively removed from metal wastewater that was diluted 10 times (Table 1). Single cell analysis only showed signals for Au^{3+} and Pd^{2+} in the sample, indicating that cells were mixed with metal wastewater (Figs. 3 and S6). This result also indicated that Au^{3+} and Pd^{2+} were selectively concentrated in cells without interference from other metals. The results of the SEM–EDS analysis also corroborated this finding (Fig. S3, Table S5). Because the pH of the diluted metal wastewater was 0.3 ± 0.1, effective recovery of Au^{3+} and Pd^{2+}, but not Pt^{4+} was achieved; this is in agreement with the results obtained using 0.4 M hydrochloric acid solution (pH 0.5; Fig. 1). However, for efficient recovery of Au^{3+} and Pd^{2+}, cells at a concentration of 7 mg/mL (dry weight) were required (Fig. S4), which is more than the concentration used in the

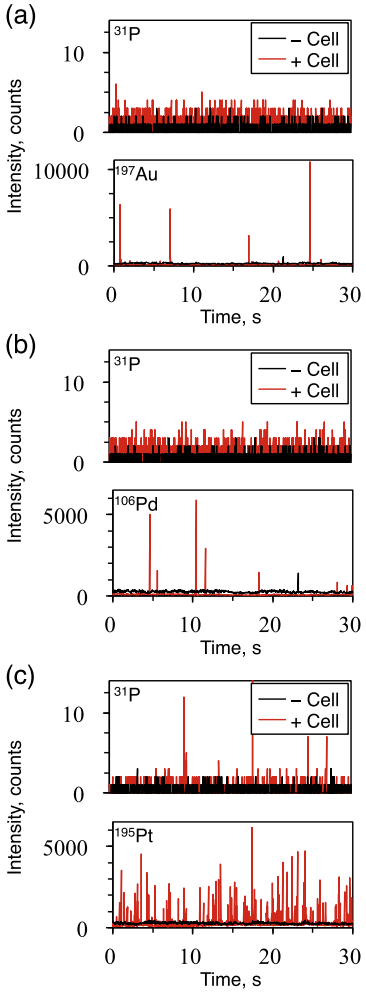

Fig. 2. Time-resolved mass spectra of *G. sulphuraria* cell-dispersed acid solution, as measured by ICP-MS. (a) ^{31}P and ^{197}Au for cells incubated with 5 mg/L Au^{3+} in 0.4 M hydrochloric acid solution (pH 0.5), (b) ^{31}P and ^{106}Pd for cells incubated with 5 mg/L Pd^{2+} in 0.4 M hydrochloric acid solution (pH 0.5), and (c) ^{31}P and ^{195}Pt for cells incubated with 0.5 mg/L Pt^{4+} in 40 mM hydrochloric acid solution (pH 2.5). Samples were diluted 100 times (a and b) or 10 times (c) with each concentration of hydrochloric acid solution and injected into the ICP-MS system directly.

experiments involving hydrochloric acid solution (Fig. 1). This is presumably because of the difference in acid quality. Pt^{4+} could be recovered from the metal wastewater upon addition of the cells and an increase in pH (up to 2.5) or with longer incubation times. However, Sn^{2+} was simultaneously recovered in the cells (Table S6). In addition, when the cells were incubated with diluted metal wastewater containing high concentrations of Au^{3+} (871 mg/L Au^{3+}, 0.86 mg/L $Fe^{2+/3+}$, 107 mg/L Cu^{2+}, 4.5 mg/L Pt^{4+}, 262 mg/L Ni^{2+}, 24 mg/L Sn^{2+}, and 0.43 M acids), the removal efficiency of Au^{3+} reached 66%, and no other contaminating metals were removed from the solution (Table S7). In summary, 34 mg of Au^{3+} was accumulated per gram of cells (dry weight) within 30 min in diluted aqua regia containing 0.4–0.5 M acid ($pH \leqslant 0.3$). This binding capacity is higher than the maximum capacity of *Spirulina platensis* at pH 4 and the binding capacity of *P. maltophilia* at pH 1 (Nakajima, 2003; Won et al., 2014), and is similar to the binding capacity of *C. vulgaris* in hydrochloric acid solution at pH 1 (Greene et al., 1986). However, aqua regia can solubilize precious metals more efficiently than hydrochloric acid solution. The binding capacity of Au^{3+} in *G. sulphuraria* is presumably higher than that in *Chlorella*. In fact, the removal efficiencies of Au^{3+} and Pd^{2+} from the same diluted metal wastewater using *Chlorella* cells were 40% and 79% (Table S8), respectively, which were lower than those obtained using *G. sulphuraria*. The higher recovery efficiency in *G. sulphuraria* may be attributed to differences in the composition of the cell wall of *Chlorella*, which mainly contains cellulose. Although it is known that the cell wall of *Cyanidium caldarium*, which belongs to the Cyanidiales family, is rich in proteins (Bailey and Staehelin, 1968), the composition of cell wall in Cyanidiales family is still unknown and it is an interesting issue in future studies.

3.3. Desorption and purification of precious metals (Au^{3+} and Pd^{2+}) from *G. sulphuraria* cells

Because efficient and selective recovery of Au^{3+} and Pd^{2+} in *G. sulphuraria* occurred through fast biosorption, it was necessary to test the desorption efficiency of these metals (Table 2). Cells retained 59 ± 7 mg/L Au^{3+} and 15 ± 1 mg/L Pd^{2+} after 15 min of incubation with diluted metal wastewater containing 57 mg/L $Fe^{2+/3+}$, 480 mg/L Cu^{2+}, 4 mg/L Pt^{4+}, 53 mg/L Au^{3+}, 46 mg/L Ni^{2+}, 5 mg/L Sn^{2+}, 12 mg/L Pd^{2+}, 11 mg/L Zn^{2+}, and 0.56 M acids. After the cells were washed with water, they were incubated in the elution solution for 30 min. As a result, 78% of Au^{3+} and 89% of Pd^{2+} were eluted using 1 M thiourea and 0.1 M HCl, while negligible elution was observed with 0.4 M HCl (Table 2). Thiourea in acidic solution forms a relatively stable complex with Au (III) and Au (I) and elutes ions efficiently (Greene et al., 1986; Wang et al., 2015). However, it also eluted trace concentrations of $Fe^{2+/3+}$, Cu^{2+}, and Pt^{4+} that were absorbed on the cell surface (Table 2). On the other hand, with the addition of 0.2 M ammonium chloride with 2.8% ammonium, 48% of Au^{3+} and 77% of Pd^{2+} were eluted from the cells without the elution of other contaminating metals. Previous studies have demonstrated that bromide strongly inhibits the biosorption of Au^{3+} compared with chloride in *C. vulgaris* (Greene et al., 1986), and that KOH is an efficient elution solution for Au^{3+} in *Sargassum natans* (Kuyucak and Volesky, 1989). In the case of *G. sulphuraria*, elution of Au^{3+} and Pd^{2+} was not affected by the replacement of chloride with bromide or sulfate (Tables 2 and S9), and elution did not occur with the addition of NH_3, KOH, or ammonium chloride at different pHs (Tables 2 and S8).

In the solution containing >0.2 M chloride (below pH 1), Au^{3+} and Pd^{2+} were mainly present as $AuCl_4^-$ and $PdCl_4^{2-}$ complexes (Nakajima, 2003; Ruiz et al., 2000). The following mechanism may explain the fast biosorption of Au^{3+} or Pd^{2+}: the positively charged amine residues under acidic conditions probably

Table 1
Recovery of Au^{3+} and Pd^{2+} from metal wastewater containing diluted aqua regia.*

	$Fe^{2+/3+}$ (mg/L)	Cu^{2+} (mg/L)	Pt^{4+} (mg/L)	Au^{3+} (mg/L)	Ni^{2+} (mg/L)	Sn^{2+} (mg/L)	Pd^{2+} (mg/L)	Zn^{2+} (mg/L)
−Cell	68 ± 8.6	379 ± 46	5.8 ± 0.6	61 ± 9	59 ± 7.5	6.5 ± 0.8	18 ± 2.0	12 ± 1.4
+Cell	63 ± 11	358 ± 60	4.7 ± 0.7	5.9 ± 2.8	58 ± 9.4	5.5 ± 0.8	0.9 ± 0.2	12 ± 1.9
Removal efficiency (%)	8	6	19	90	0.3	16	95	6

Values are averages ± S.D.
* 0.56 M acids.

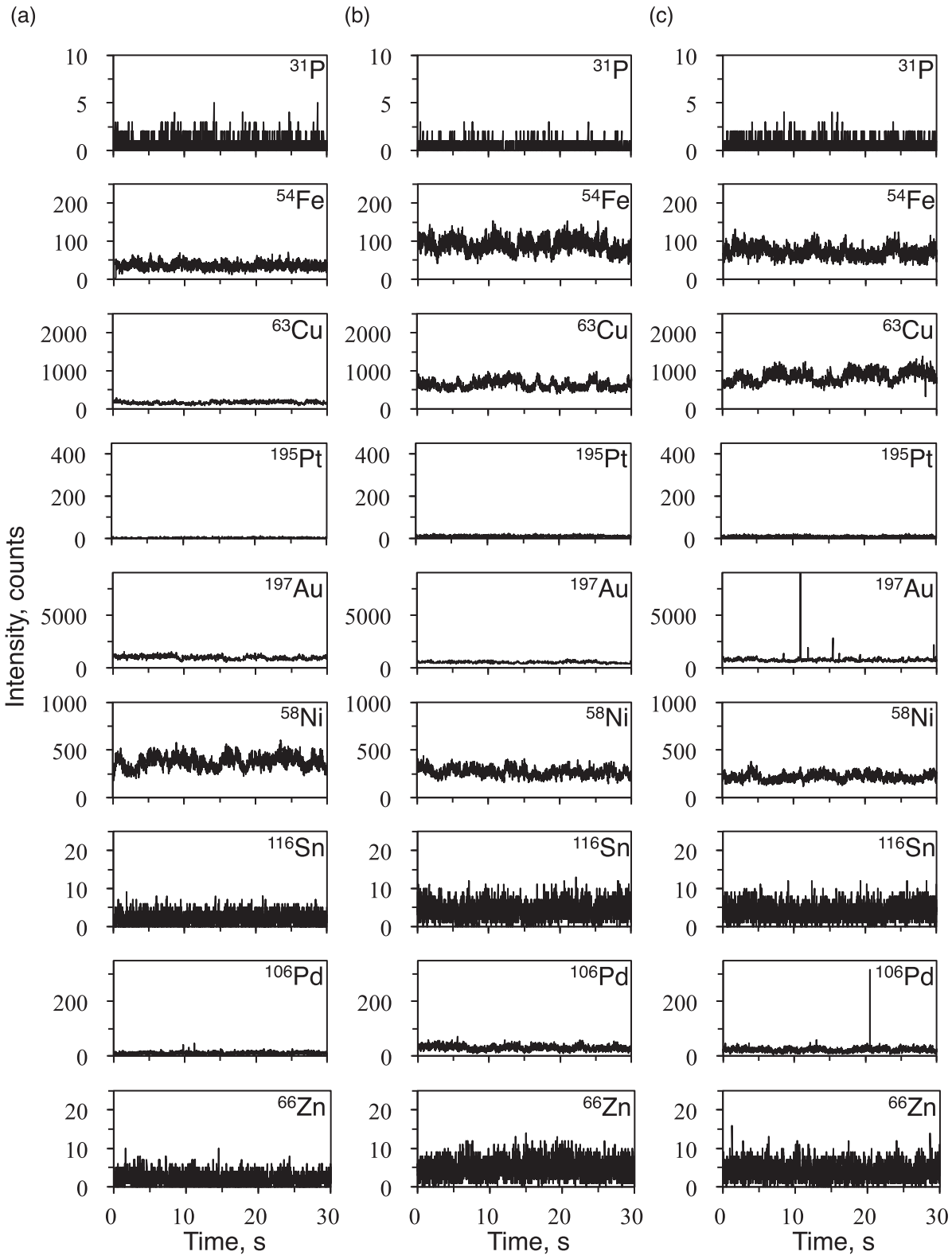

Fig. 3. Time-resolved mass spectra (^{31}P, ^{54}Fe, ^{63}Cu, ^{195}Pt, ^{197}Au, ^{58}Ni, ^{116}Sn, ^{106}Pd, and ^{66}Zn) of (a) *G. sulphuraria* cell-dispersed acid solution, (b) diluted aqua regia-based metal wastewater, and (c) *G. sulphuraria* cell-dispersed aqua regia-based metal wastewater, as measured by ICP-MS. Diluted aqua regia-based metal wastewater contains 57 mg/L $Fe^{2+/3+}$, 480 mg/L Cu^{2+}, 4 mg/L Pt^{4+}, 53 mg/L Au^{3+}, 46 mg/L Ni^{2+}, 5 mg/L Sn^{2+}, 12 mg/L Pd^{2+}, 11 mg/L Zn^{2+} and 0.56 M acids. Samples were diluted 500 times with diluted aqua regia containing 0.5 M acids and injected into the ICP-MS directly.

interacted with the complexes at the surface of the biosorbents. Static characterization and inhibitor experiments have supported this model in many microorganisms and biosorbents (Greene et al., 1986; Wang et al., 2015). $AuCl_4^-$ and $PdCl_4^{2-}$ were presumably removed as soluble complexes from the surface of *G. sulphuraria* cells with the addition of 0.2 M ammonium chloride with 2.8% ammonium. It is possible that Au^{3+} or Pd^{2+} formed complexes with ammonium ions as $Au(NH_4)^+$ or $Pd(NH_4)^{2+}$ by ion exchange.

Table 2
Elution of Au^{3+} and Pd^{2+} from cells retaining 59 ± 7 mg/L Au^{3+} and 15 ± 1 mg/L Pd^{2+} after incubation with metal wastewater* for 15 min.

Elution solution	Au^{3+} (mg/L)	Pd^{2+} (mg/L)	$Fe^{2+/3+}$ (mg/L)	Cu^{2+} (mg/L)	Pt^{4+} (mg/L)	Ni^{2+} (mg/L)	Sn^{2+} (mg/L)	Zn^{2+} (mg/L)
0.4 M HCl	2.4 ± 0.8	0.0 ± 0.0	2.2 ± 2.2	11.6 ± 0.1	ND	ND	0.2 ± 0.0	ND
0.2 M NH_4Br, 2.8% NH_3 (pH11)	29.2 ± 2.3	11.6 ± 0.7	ND	3.8 ± 3.8	ND	ND	ND	ND
0.2 M NH_4Cl, 2.8% NH_3 (pH11)	28.2 ± 0.3	11.3 ± 0.3	ND	ND	ND	ND	ND	ND
0.1 M KOH	3.9 ± 1.6	0.4 ± 0.1	ND	3.8 ± 3.8	ND	ND	0.4 ± 0.1	ND
1 M Thiourea, 0.1 M HCl	46.2 ± 6.4	13.4 ± 1.2	5.5 ± 2.8	11.6 ± 0.1	0.6 ± 0.0	ND	ND	ND

Values are averages ± S.E.
* 57 mg/L $Fe^{2+/3+}$, 480 mg/L Cu^{2+}, 4 mg/L Pt^{4+}, 53 mg/L Au^{3+}, 46 mg/L Ni^{2+}, 5 mg/L Sn^{2+}, 12 mg/L Pd^{2+}, 11 mg/L Zn^{2+} and 0.56 M acid.

However, no sediment was formed with the addition of hydrochloric acid to the elution solution, although the solubility products of the ammonium complexes of Au^{3+} or Pd^{2+} are small. Thus, Au^{3+} and Pd^{3+} are probably solubilized as $AuCl_4^-$ and $PdCl_4^{2-}$ in the elution solution containing 0.2 M ammonium chloride 2.8% ammonium. These complexes can be separated easily by using conventional methods (e.g. solvent extraction) for further purification.

4. Conclusion

G. sulphuraria recovered precious metals with high efficiency through biosorption, similar to other microorganisms known to be high accumulators. Over 90% of Au^{3+} and Pd^{2+} were selectively recovered from aqua regia-based metal wastewater containing 10-fold higher concentrations of Cu^{2+} than those of Au^{3+} and Pd^{2+}. Ammonium salt solution (0.2 M) with 2.8% ammonium eluted Au^{3+} and Pd^{2+} as useful complexes for conventional purification. Because the entire process was completed within 1 h, the use of *G. sulphuraria* has promising applications in the recovery of low concentrations of precious metals from industrial wastewater that has not been recycled chemically or pyrometallurgically.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2016.01.061.

References

Allen, M.B., 1959. Studies with *Cyanidium caldarium*, an anomalously pigmented chlorophyte. Arch. Microbiol. 32, 270–277.
Ascione, R., Southwick, W., Fresco, J.R., 1966. Laboratory culturing of a thermophilic alga at high temperature. Science 12, 752–755.
Bailey, R.W., Staehelin, L.A., 1968. The chemical composition of isolated cell walls of *Cyanidium caldarium*. J. Gen. Microbiol. 54, 269–276.
Das, N., 2010. Recovery of precious metals through biosorption — a review. Hydrometallurgy 103, 180–189.
Fukuda, I., 1958. Physiological studies on a thermophilic blue green alga, *Cyanidium caldarium* Geitler. Bot. Mag. Tokyo. 71, 79–86.
Godlewska-Żyłjuewucz,, 2003. Biosorption of platinum and palladium for their separation/preconcentration prior to graphite furnace atomic absorption spectrometric determination. Spectrochim. Acta part B 58, 1531–1540.
Graverholt, O.S., Eriksen, N.T., 2007. Heterotrophic high-cell-density fed-batch and continuous-flow cultures of *Galdieria sulphuraria* and production of phycocyanin. Appl. Microbiol. Biotechnol. 77, 69–75.
Greene, B., Hosea, M., McPherson, R., Henzi, M., Alexander, M.D., Darnall, D.W., 1986. Interaction of Gold (I) and Gold (III) complexes with algal biomass. Environ. Sci. Technol. 20, 627–632.
Gross, W., Schnarrenberger, C., 1995. Heterotrophic growth of two strains of the acido-thermophilic red alga, *Galdieria sulphuraria*. Plant Cell Physiol. 36, 633–638.
Kuyucak, N., Volesky, B., 1989. The elution of gold sequestered on a natural biosorbent. Biorecovery 1, 205–218.
Mack, C., Wilhelmi, B., Duncan, J.R., Burgess, J.E., 2007. Biosorption of precious metals. Biotechnol. Adv. 25, 264–271.
Minoda, A., Sakagami, R., Yagisawa, F., Kuroiwa, T., Tanaka, K., 2004. Improvement of culture conditions and evidence for nuclear transformation by homologous recombination in a red alga, *Cyanidioschyzon merolae* 10D. Plant Cell Physiol. 45, 667–671.
Minoda, A., Sawada, H., Suzuki, S., Miyashita, S., Inagaki, K., Yamamoto, T., Tsuzuki, M., 2015. Recovery of rare earth elements from the sulfo-thermophilic red alga *Galdieria sulphuraria* using aqueous acid. Appl. Microbiol. Biotechnol. 99, 1513–1519.
Miyashita, S., Groombridge, A.S., Fujii, S., Minoda, A., Takatsu, A., Hioki, A., Chiba, K., Inagaki, K., 2014. Highly efficient single-cell analysis of microbial cells by time-resolved inductively coupled plasma mass spectrometry. J. Anal. At. Spectrom. 29, 1598–1606.
Nagasaka, S., Nishizawa, N.K., Watanabe, T., Mori, S., Yoshimura, E., 2003. Evidence that electron-dense bodies in *Cyanidium caldarium* have an iron-storage role. Biometals 16, 465–470.
Nakajima, A., 2003. Accumulation of gold by microorganisms. World J. Microbiol. Biotechnol. 19, 369–374.
Ruiz, M., Saste, A.M., Guibal, E., 2000. Palladium sorption on glutaraldehyde-crosslinked chitosan. React. Funct. Polym. 45, 155–173.
Selavaratnam, T., Pegallapati, A.K., Montelya, F., Rodriguez, G., Nirmalakhandan, N., Voorhies, W.V., Lammers, P.J., 2014. Evaluation of a thermo-tolerant acidphilic alga, *Galdieria sulphuraria*, for nutrient removal from urban wastewaters. 2014. Bioresour. Technol. 156, 395–399.
Umali, L.J., Duncan, J.R., Burgess, J.E., 2006. Performance of dead *Azolla filiculoides* biomass in biosorption of Au from wastewater. Biotechnol. Lett. 28, 45–49.
Umeda, H., Sasaki, A., Takahashi, K., Haga, K., Takasaki, Y., Shibayama, A., 2011. Recovery and concentration of precious metals from strong acidic wastewater. Mater. Trans. 52, 1462–1470.
Wang, J., Wei, J., Li, J., 2015. Rice straw modified by click reaction for selective extraction of noble metal ions. Bioresour. Technol. 177, 182–187.
Won, S.W., Kotte, P., Wei, W., Lim, A., Yun, Y.-S., 2014. Biosorbents for recovery of precious metals. Bioresour. Technol. 160, 203–212.
Yoshimura, E., Nagasaka, S., Satake, K., Mori, S., 2000. Mechanism of aluminium tolerance in *Cyanidium caldarium*. Hydrobiologia 433, 57–60.
Żyłjuewucz, B., Kozłowska, M., 2005. Solid phase extraction using immobilized yeast *Saccharomyces cerevisiae* for determination of palladium in road dust. Anal. Chim. Acta 539, 61–67.