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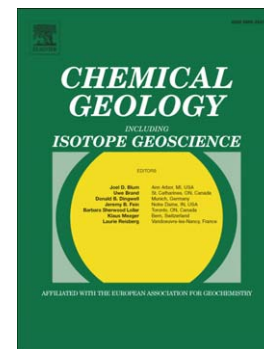
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The effect of iron-oxidising bacteria on the stability of gold (I) thiosulphate complex

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Running Title: Iron-oxidising bacteria affect gold (I) thiosulphate stability

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Abstract

An acidophilic, iron-oxidising bacterial consortium was collected from Rio Tinto near Berrocal, Spain. This primary enriched culture was used to examine the effect of acidophilic iron-oxidising bacteria on the stability of soluble gold (I) thiosulphate. Stationary phase cultures and separate components of the cultures (i.e., aqueous ferric iron, iron oxyhydroxide precipitates and non-mineralised bacterial cells) were exposed to gold (I) thiosulphate solutions forming different experimental-gold systems. These experimental systems rapidly removed gold from solutions containing 0.002 mM – 20 mM gold thiosulfate. Scanning and transmission electron microscopy demonstrated that the different culture fractions immobilised gold differently: the entire bacterial culture-gold systems precipitated 100 nm-size gold colloids; aqueous ferric iron-gold systems precipitated colloidal gold sulphide that ranged in diameter from 200 nm to 2 μ m; iron oxyhydroxide-gold systems precipitated 5 nm-size gold sulphide colloids; and the bacteria-gold systems precipitated gold colloids ~2 nm in size along the bacterial cell envelope. Aqueous and solid ferric iron was critical in the destabilisation of the gold (I) thiosulphate complex. Analysis of the entire bacterial culture-, aqueous ferric iron- and iron oxyhydroxide-gold systems exposed to 2 mM gold using X-ray absorption near edge spectroscopy demonstrated that Au^+ was immobilised from solution as gold sulphide (Au_2S). The reaction between iron-oxidising bacteria and their ferric iron by-products with gold (I) thiosulphate demonstrated that thiosulphate ions would be an unstable gold complexing ligand in nature. Gold (I) thiosulphate is intuitively transformed into nanometer-scale gold sulphide or elemental gold within natural, acidic weathering

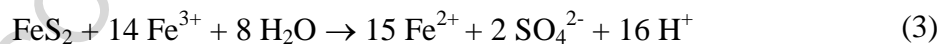
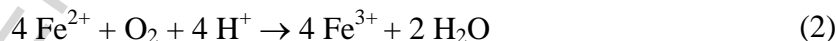
environments with the potential to precipitate gold in jarosite that can subsequently be preserved in gossans over geological time.

Keywords: gold (I) thiosulphate, gold sulphide, colloidal gold, biogeochemistry, iron-oxidising bacteria

1. Introduction

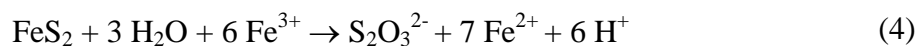
Physical and chemical weathering of massive sulphide deposits leads to the oxidation of iron sulphide minerals and results in near-surface environmental conditions characterised by increased acidity and aqueous ferric iron and sulphate concentrations (Blowes and Jambor, 1990; Nordstrom and Southam, 1997; Nordstrom and Alpers, 1999; Lopez-Archilla et al., 2001; Levings et al., 2005; Ferreira da Silva et al., 2006; Alvarez-Valero et al., 2008; Fernandez-Remolar et al., 2008). These environments, broadly referred to as acid rock drainage (ARD) environments, are influenced by physical factors such as surrounding geology, topography, climate and water table levels that contribute to weathering processes (Webster and Mann, 1984; Dold and Fontbote, 2001; Nordstrom, 2009; Reaction 1). Many studies have demonstrated that the oxidation of sulphide minerals, e.g., pyrite, chalcopyrite and arsenopyrite, under aqueous conditions generally involves either chemical or electrochemically catalysed reactions (see Steger and Desjardin, 1978; Lawson, 1982; Nicholson et al., 1988; Buckley and walker, 1988; Nesbitt et al., 1995; Nesbitt and Muir, 1998; Rimstidt and Vaughn, 2003; Chandra and Gerson, 2010). While oxidation of these sulphides typically involve abiotic mechanisms, Singer and Stumm (1970) demonstrated that increased acidity and soluble ferric iron

concentrations must be attributed to microbial metabolic activity. Acidophilic and acidotolerant chemolithoautotrophic bacteria are ubiquitous in ARD weathering environments where sulphide-bearing minerals provide ideal substrates for bacterial growth (Ferris et al., 1988; Konhauser, 1998; Gonzalez-Toril et al., 1999; Ferris et al., 2004; Fowler and Crundwell, 1999; Hedirch et al., 2011). Iron-oxidising bacteria such as *Acidithiobacillus ferrooxidans* contribute to the rapid solubilisation of iron sulphide minerals by oxidising ferrous iron to ferric iron which in turn rapidly oxidises pyrite relative to abiotic weathering rates (Singer and Stumm, 1970; Reactions 2 and 3). In these environments, ferrous iron oxidation rates are likely to occur somewhere between the abiotic rate and the optimal rate since metabolic growth of iron-oxidising bacteria are influenced by other environmental factors such as essential nutrients, hydrological activity and pH (Nordstrom and Southam, 1997).



The “physical state” of gold in near-surface weathering environments is considered to be dynamic (Wilson, 1984; Webster and Mann, 1984). In particular, the contribution of the biosphere to the establishment of acidic, oxidising environments is not only important for the physical and chemical weathering of metal sulphides but could also lead to the mobility of gold as soluble complexes (Plyusnin et al., 1981). Massive sulphide ore deposits, such as the Iberian Pyrite Belt of southern Spain and Portugal, can host primary gold of economic significance (Huston and Large, 1989; Hutchinson, 1990; Large, 1990; Huston et al., 1992; Leistel et al., 1998). However, enriched secondary gold

dispersion halos have also been observed in supergene enrichment environments and in gossans (Spurr and Garrey, 1908; McHuge, 1984, 1988; Freyssinet et al., 1989; Boyle, 1979; Colin et al., 1997; Hough et al., 2008). Supergene deposits can contain 1 to 3 g/t gold (Basciano and Peterson, 2008; Strauss and Beck, 1999). Sulphur-bearing ligand ions, such as thiosulphate, are formed during the oxidative weathering of metal sulphides and are thought to be an important gold complexing agent in these systems (Puddephatt, 1978; Plyusnin et al., 1981; Mann, 1984; Webster, 1985; Renders and Seward, 1989; Bendetti and Boulegue, 1991; Bowell, 1992; Reaction 4). In these environments, thiosulphate ions are common ligands contributing to soluble gold mobility as stable complexes under acidic, oxidising conditions when excess sulphur is present. These complexes are intuitively transported within surficial hydrological systems (Webster 1985; Bowell, 1992; Reaction 5). The formation of gold complexes with other oxidised sulphur ligands such as sulphite (SO_3^{2-}), sulphate (SO_4^{2-}) and tetrathionate ($\text{S}_4\text{O}_6^{2-}$) have not been observed in natural environments and are therefore considered meta-stable, transitory ligands (Bowell, 1992; Aylmore and Muir, 2001). It is important to note that sulphur-oxidising bacteria can also contribute to the formation of thiosulphate ions (Lakin et al., 1974).



Many field and experimental studies on gold mobility and dispersion have focused on the adsorption and co-precipitation of gold on iron oxyhydroxide minerals formed from ferric iron-bearing solutions leading to supergene enrichment (Andrade et al., 1991; Machensky et al., 1991; Schoonen et al., 1992; Greffie et al., 1996; Uchida et

al., 2002). However, as Freise (1931) first proposed, organic substances could act as reducing agents for soluble gold complexes resulting in the formation of secondary gold. Goldschmidt (1937) contributed to this idea by suggesting that observed metal concentrations within natural environments could be attributed to bacterially catalysed geochemical processes. Recent studies have focused specifically on the biogeochemical cycling of gold in near-surface environments (see Southam and Beveridge, 1994, 1996; Lengke et al., 2006a,b, 2007; Reith and McPhail, 2006; Reith et al., 2006, 2007; Kenney et al., 2012; Song et al., 2012, Fairbrother et al., 2012). Collectively, these studies demonstrated the affects of various bacteria on the immobilisation of gold from soluble aurous and auric complexes through both passive and active gold biomineralisation. An unanswered question, however, is the “fate” of soluble gold complexes in oxidised, weathering environments where high iron concentrations and acidic conditions are sustained by active metabolism of chemolithoautotrophic bacteria and supergene conditions dominated by gossan minerals. Therefore, the purpose of this present study was to examine the stability of gold (I) thiosulphate when exposed to acidophilic, iron-oxidising bacteria and the associated geochemical conditions induced by this physiological group of bacteria.

2. Materials and Methods

2.1 Sample acquisition

Surfaces of cobbles in the Rio Tinto river, Spain are known to have iron oxyhydroxide mineral coatings (i.e., goethite, jarosite and hematite; see Fernandez-Remolar et al., 2005) that contain a consortium of preserved and metabolically active

iron-oxidising bacteria (Preston et al., 2011). Iron oxyhydroxide coatings along with river water were sampled (37° 35' 33.27" N, 6° 33' 1.84" W) using sterile, 50 mL Luer-Lok™ syringes and placed in 15 mL Falcon tubes. This site was chosen for sampling because it is proximally located near historic gold-bearing mines associated with gossans (Leistel et al., 1998) and the geochemical conditions of the river support a diverse range of acidophilic microorganisms (Lopez-Archilla et al., 2001; Amaral et al., 2002; Sabater et al., 2003; Gonzalez-Toril et al., 2003).

Sampled iron oxyhydroxide mineral precipitates were homogenised by vortex using a Wisemix® Instrument to disperse bacterial cells in the Rio Tinto sample. A bacterial cell count of this homogenised sample was performed using phase contrast light microscopy and a Petroff-Hausser counting chamber.

2.2 Bacterial cultures

Enriched bacterial cultures of the iron-oxidising consortium were produced by inoculating 0.5 mL of the homogenised iron oxyhydroxide sample into 4.5 mL of modified media defined by Silverman and Lundgren (1959). The modified media contained 39.5 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, pH adjusted to 2.3 using 2 M sulphuric acid and was filter sterilised using a 0.45 μm pore-size filter. Medium without a bacterial inoculum will be referred to as “fresh media” for simplicity here after. Bacterial cultures were incubated at room temperature (approximately 22°C) for three weeks in Fisherbrand® 13 × 100 mm borosilicate glass test tubes with plastic push caps to maintain aerobic conditions. Growth to stationary phase (3 weeks) was indicated by a well-defined phenotype of iron oxyhydroxide mineral precipitates coating the inside surface of the test

tubes. A bacterial cell count was performed after three weeks of incubation using the Most Probable Number (MPN) statistical method described by Cochran (1950). The pH and Eh of the enriched bacterial cultures were also measured using a Denver Instrument Basic pH/Eh Meter with an electrode calibrated to pH 2 and 4 reference standards using potassium biphthalate buffer and ZoBell's solution (Nordstrom, 1977), respectively. Uncertainties of pH measurements were ± 0.03 pH units and Eh measurements were ± 0.05 V.

2.3 X-ray diffraction of iron oxyhydroxide mineral precipitate

Iron oxyhydroxide mineral precipitates that formed on the inside surface of the borosilicate glass test tube of the bacterial cultures were rinsed (see section 2.5.4) and collected on a 0.45 μm pore-size filter. The rinsed mineral precipitates were air-dried at room temperature for 24 hours. The dehydrated mineral precipitates were then pulverized using a sterile mortar and pestle to obtain a fine powder. Approximately 200 mg of the powdered iron oxyhydroxide mineral precipitate was back-pack mounted into a well (200 mm \times 150 mm \times 0.03 mm) of a glass slide to reduce surface roughness and preferential orientation of particles. A Rigaku diffractometer (Powder and Micro X-ray Diffraction Laboratory, Western University) operating at 35 kV and 45 mA with a cobalt anode source (Co $K\alpha$, $\lambda = 1.78897 \text{ \AA}$) was used to collect qualitative powdered X-ray diffraction (pXRD) data from 10° to 90° 2θ with a 0.05° step-size and a step speed of 1 second. Bruker AXS EVA software (BrukerAXS, 2005) and the International Center for Diffraction Data (ICDD) PDF-4 database was used to analyse the generated diffractogram.

2.4 Gold stock solutions

Sodium gold (I) thiosulphate of 99.9% purity was purchased from Alfa Aesar and dissolved in distilled, deionised water to form a calculated 40 mM gold stock solution. The solution was filter-sterilised using a 0.1 μm pore-size filter and made into four ten-fold serial dilutions each with a final concentration of 0.02, 0.2, 2 and 20 mM gold. The purpose of using the higher gold concentrations was to enable detection using scanning and transmission electron microscopy and synchrotron-based analysis of experimental-gold systems.

2.5 Experimental-gold systems

Enriched cultures of the iron-oxidising bacterial consortium and each separate component of the culture, i.e., aqueous ferric iron, iron oxyhydroxide mineral precipitates and bacteria, were exposed to gold stocks creating different experimental-gold systems to examine the direct and indirect affects of iron-oxidising bacteria on gold (I) thiosulphate stability (see section 2.5.1 to 2.5.6). All experimental-gold systems were homogenised by a vortex then wrapped in aluminium foil to prevent any photocatalytic effects once gold stocks were added. Each type of experimental-gold system was performed in triplicate. Passing experimental-gold systems through separate 0.1 μm pore-size filters removed all solid material and stopped the exposure reaction. The pH and Eh of all experimental-gold systems were measured in the same manner described for the enrichment of bacterial cultures. Solid filtrates were analysed using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Filtered solutions

were analysed for un-reacted, aqueous gold using a Perkin-Elmer Optima 3300-DV Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES) at the Analytical Chemistry Facility, Biotron, Western University. The difference in concentration between the gold stocks and the measured, un-reacted gold was calculated to obtain the amount of gold immobilised from the fluid phase of each experimental-gold system.

2.5.1 Stationary phase bacterial culture-gold system: Gold stocks were added to the primary bacterial cultures to produce final gold concentrations equalling 0.002, 0.02, 0.2, 2 and 20 mM. The entire bacterial culture-gold system possessing 0.002 mM gold was analogous to gold concentrations found in natural waters (McHugh, 1988). Each bacterial culture-gold system was allowed to react for 1, 2, 4, 8 and 24 hours.

2.5.2 Spent media-gold system: Iron-oxidising bacteria produce a high concentration of soluble ferric iron as a by-product of their active metabolism via ferrous iron oxidation (Singer and Stumm, 1970). Stationary phase bacterial cultures were filtered to remove solid components, e.g., iron oxyhydroxide mineral precipitates and the bacterial consortium, leaving only the fluid phase containing soluble iron. The filtered fluid phase of bacterial culture is hereafter referred to as “spent media”. A 40 mM gold stock was added to an equal volume of spent media (1:1 ratio) forming a spent media-gold system with a final concentration of 20 mM gold. This system was allowed to react for 24 hours.

2.5.3 Fresh media-gold system: Spent media may contain residual, non-metabolised ferrous iron even after three weeks incubation. Therefore, a 1:1 ratio of a 40 mM gold

stock was added to fresh media forming a fresh media-gold system with an overall concentration of 20 mM gold. This fresh media-gold system was complimentary to the spent media-gold system because its purpose was to determine whether or not residual, non-metabolized ferrous sulphate would react with gold (I) thiosulphate after 24 hours of exposure.

2.5.4 Iron oxyhydroxide-gold system: Active metabolism of iron-oxidising bacteria contributes to the precipitation of solid iron oxyhydroxide minerals (Singer and Stumm, 1970). Spent media, along with any bacteria occurring in this fluid phase of the enriched bacterial cultures were discarded leaving iron oxyhydroxide mineral precipitates attached to the inner surface of the borosilicate glass tubes. Sterile, distilled, deionised water was added to the tubes so that the mineral precipitates could be removed from the glass surface using the vortex mixer. The mineral precipitates were then filtered and re-suspended in 1 mL of sterile, distilled, deionised water to rinse out any spent media. The rinsed mineral precipitates were then centrifuged for 1 minute at $12,000 \times g$ to form a pellet and the supernatant was discarded. This rinse procedure was performed three times. After the final rinse and the supernatant was discarded, a 20 mM gold stock was added to the pellet forming an iron oxyhydroxide-gold system that was allowed to react for 24 hours.

2.5.5 Bacterial-gold system: Spent media was removed from enriched bacterial cultures, pooled and centrifuged for 1 minute at $12,000 \times g$ to obtain a pellet of iron-oxidising bacterial cells from the fluid phase. The supernatant was discarded and the pellet was

suspended in 1 mL of sterile, distilled, deionised water and homogenised. A bacterial cell count of approximately 2.4×10^5 bacteria/mL was determined using the same method described for the original iron-oxidising bacterial sample (see section 2.1). The bacterial suspension was re-centrifuged to form a pellet and was then rinsed three times in the same manner and purpose described in section 2.5.4. After the final rinse procedure and the supernatant was discarded, a 20 mM gold stock was added to the pellet forming a bacterial-gold system that was allowed to react for 24 hours.

2.5.6 Chemical control: Each gold stock solution was also used as a chemical control. The purpose of this chemical control was to determine whether reduction of gold (I) thiosulphate could occur without the presence of any perturbation over 24 hours.

2.6 Scanning electron microscopy-energy dispersive spectroscopy

Enriched bacterial cultures, filtrates from stationary phase bacterial cultures-gold systems and spent media-gold systems exposed to 20 mM gold for 24 hours were prepared for Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDS). The filtrates were fixed for 2 hours with 2%_(aq) glutaraldehyde, dehydrated in sequential, aqueous 25, 50, 75 and 3 × 100%_(aq) ethanol series, critical-point dried using a Tousimis Research Corporation Samdri-PVT-3B critical-point drier, and mounted onto separate 12 mm aluminium stubs with carbon adhesive tabs. A Denton Vacuum Desk II sputter coater was used to coat the filtrates with a 3 nm thick osmium deposition to reduce charging during SEM-EDS analysis. A LEO Ziess 1540XB Field Emission Gun-Scanning Electron Microscope (FEG-SEM) equipped with an Oxford Instruments'

INCAx-sight Energy Dispersive Spectrometer (EDS) (Western Nanofabrication Facility) operating at 3 kV or 10 kV accelerating voltage was used for imaging and semi-quantitative elemental composition, respectively.

2.7 Transmission electron microscopy-energy dispersive spectroscopy

Separate pellets of iron oxyhydroxide mineral precipitate and bacteria were used as a comparison for filtrates from iron oxyhydroxide- and bacteria-gold systems exposed to 20 mM gold for 24 hours. All four samples were prepared for Transmission Electron Microscopy-Energy Dispersive Spectroscopy (TEM-EDS) when fixed for 2 hours in 2%_(aq) glutaraldehyde, enrobed in 2%_(wt/vol) noble agar, dehydrated in a 25, 50, 75 and 3 × 100%_(aq) acetone series and embedded in Epon plastic (Graham and Beveridge, 1990). The embedded samples were cut to 70 nm ultrathin sections using a Reichert-Jung Ultracut E ultramicrotome and collected on a Formvar-carbon coated, 200-square mesh copper grids. The sections were examined using a Phillips CM-10 Transmission Electron Microscope (TEM) (Imaging and Data Analysis Facility, Biotron, Western University) operating at 80 kV. Qualitative elemental analysis was determined using a Philips 420 TEM equipped with EDS Genesis x-ray microanalysis system (Imaging and Data Analysis Facility, Biotron, Western University) operating at the same voltage.

2.8 X-ray absorption near-edge spectroscopy

The stationary phase bacterial culture-, spent media- and iron oxyhydroxide-gold systems exposed to 20 mM gold for 1 hour were used for X-ray Absorption Near-Edge Spectroscopy (XANES) to differentiate possible intermediate gold oxidation states. Each

experimental-gold system was centrifuged and rinsed with deoxygenated, deionised water in the same manner described for preparation of iron oxyhydroxide- and bacterial-gold systems. The rinsed experimental-gold systems formed wet pastes that were separately sealed in an acid resistant, Teflon fluid cell (3.5 cm × 1 cm × 0.5 cm) with Kapton film. Five to seven scans of each fluid cell were collected in fluorescence mode using a Canberra solid-state germanium, multi-elemental detector and were averaged at beamline 9BM CMC-XOR-Sector 9, Advanced Photon Source, Argonne National Laboratory. All spectral scans were monitored for induced radiation effects during data acquisition although none were detected. The Si (III) monochromator was calibrated to the gold edge (11919 eV) using the first peak of the first derivative XANES spectrum of a metallic gold foil standard. Energy scales for each fluid cell, containing an experimental system, were referenced to the gold foil spectrum.

Three gold (I) compounds and metallic gold foil each with 99.9% purity were purchased from Alfa Aesar and used as reference standards. Gold (I) thiosulphate and gold (I) thiomalate were dissolved in distilled, deionised water forming separate 20 mM gold solutions. Solid gold (I) sulphide was ground with a sterile mortar and pestle and a fine monolayer was deposited on Kapton tape. XANES spectra of these gold standards were measured in transmission mode using an ionisation chamber filled with nitrogen gas at 1 atm while elemental gold was measured simultaneously with the experimental-gold systems. The different standards represented potential gold complexes that could form during gold (I) thiosulphate destabilisation when exposed to the stationary phase iron-oxidising consortium and each separate component of the culture.

2.9 X-ray absorption near-edge spectroscopy analysis

Athena 8.054 (Ravel and Newville, 2005) was used to process the XANES data. A linear regression was fitted to the pre-edge, e.g., -150 to -75 eV relative energy, and post-edge, e.g., 150 and 300 eV, regions of the XANES spectra in order to subtract the pre-edge baseline and to apply XANES normalisation.

3. Results

3.1 Bacterial cultures

Positive growth, based on the MPN statistical analysis, was demonstrated by the increased number of cells (4.8×10^5 bacteria/mL) in the enriched bacterial cultures relative to the original sample (7×10^3 bacteria/mL). Evidence of active, iron-oxidising bacterial metabolism was indicated by increased acidity of 2.24 from 2.3, increased Eh of 0.73 V from 0.65 V and the formation of a red-orange, iron oxyhydroxide mineral precipitate that coated the inside surface of the borosilicate test tubes (Fig. 1, inset). It should be noted that the pH of the spent media was the same as the enriched bacterial cultures (pH = 2.24).

3.2 pXRD analysis of iron oxyhydroxide mineral precipitate

Analysis of the pXRD diffractogram indicated that the iron oxyhydroxide mineral precipitate was composed of both ammoniojarosite ($(\text{NH}_4)\text{Fe}_3(\text{SO}_4)_2(\text{OH})_6$) and hydroniumjarosite ($(\text{H}_3\text{O})\text{Fe}_3(\text{SO}_4)_2(\text{OH})_6$) (Fig. 2). Here after, the reference to iron oxyhydroxide mineral precipitate will be called jarosite.

3.3 Aqueous chemical analysis

The reduction of gold (I) thiosulphate was observed in the entire enriched bacterial culture-gold systems as the fluid phase gradually changed from red-orange to clear and the jarosite turned slightly brown. Calculations of immobilised gold, inferred from ICP-AES analysis (Table 1), indicated that greater than 90% of soluble gold in the entire enriched bacterial culture-gold systems was removed from solution within the first hour of exposure and increased with time. More importantly, the entire bacterial culture-gold systems exposed to 20 mM gold for 24 hours removed more gold from solution in comparison to the jarosite- (31%) and bacterial-gold systems (4%). Spent media-gold systems, however, removed approximately the same amount of gold as the entire enriched bacterial culture-gold system. Rapid removal of gold (I) thiosulphate from solution was observed as an opaque, black precipitate that formed in suspension. The precipitate settled to the bottom of the borosilicate glass tube within minutes leaving the solution completely clear. Fresh media-gold systems and the chemical controls had no observed colour change or precipitate formation indicating that gold was not removed from solution. Detailed chemical analysis of each experimental-gold system is found in Table 1.

3.4 SEM-EDS analysis

The enriched bacterial cultures contained nanometre-scale, acicular jarosite mineral precipitates that formed micrometre-scale sheets and spherical structures with rod-shaped bacteria occurring on the surface (Fig. 3a). Filtrates from entire enriched bacterial cultures-gold systems contained the same jarosite morphology with bacteria.

However, soluble gold was immobilised as 100 nm gold sulphide colloids forming clusters on the surface of jarosite mineral precipitates (Fig. 3b). Filtrates from spent media-gold systems demonstrated that soluble gold was immobilised as colloidal precipitates ranging from 200 nm to 2 μm in diameter and were also composed of gold and sulphur (Fig. 4). These colloids formed larger clusters and the gradual formation explains the observed black precipitate in suspension in the spent media-gold system.

3.5 TEM-EDS analysis

High-resolution TEM demonstrated that jarosite occurred as aggregated spheres 100 nm in size and composed of radial acicular structures (Fig. 5a). When exposed to 20 mM gold for 24 hours, the jarosite-gold system formed 2 nm colloids composed of gold and sulphur (see section 3.6). It is important to note that the filamentous appearance of jarosite diminished as the precipitation of gold sulphide colloids replaced the acicular minerals (Fig. 5b). Bacteria obtained from the fluid phase of enriched cultures contained no evidence of iron mineralisation (Fig. 6a). However, bacteria from bacterial-gold systems demonstrated extensive extracellular gold mineralisation. Reduction of soluble gold (I) thiosulphate from solution occurred as colloidal gold approximately 2 nm in diameter (Fig. 6b). Although the cell wall structure was not observed, the extensively gold-mineralised extracellular surface preserved the bacterial cell shape (Fig. 6c).

3.6 XANES analysis

The XANES spectra of the four relevant gold compounds are illustrated in Figure 7. Unlike characteristic peaks of the elemental gold standard, gold sulphide and gold (I)

thiosulphate standards have broad spectral features occurring after the L-III absorption edge. The XANES spectra generated from the entire enriched bacterial cultures-, spent media- and jarosite-gold systems exposed to 20 mM gold for 1 hour confirmed the precipitation of gold sulphide from the reduction of gold (I) thiosulphate (Fig. 7).

4. Discussion

Previous studies have demonstrated that active metabolism of iron-oxidising bacteria, e.g., *A. ferrooxidans*, form jarosite-group minerals that are stable under oxidised, highly acidic, iron- and sulphur-rich conditions. The rate of iron oxidation by bacteria and the presence of other organic material, i.e., extracellular polymeric substance, are critical factors that contributed to the formation of different crystal morphologies of jarosite-group minerals (see Brown, 1971; Sasaki et al., 1995; Sasaki and Konno, 2000; Chan et al., 2009). In this study, ammoniojarosite and hydroniumjarosite formed as nanometre-size, acicular mineral precipitates within enriched bacterial cultures (Fig. 1 inset, 3a). Ammonium sulphate was a constituent of the media defined by Silvermann and Lundgren (1959) and NH_4^+ was the univalent cationic end member for ammoniojarosite. Similarly, highly acidic spent media likely provided H_3O^+ as the alternate end member forming hydroniumjarosite. The pXRD diffractogram (Fig. 2) had slight peak shifts for hydroniumjarosite and could be attributed to the deformation of crystal lattice structure when the samples were air-dried. Nonetheless, the identification of jarosite, from the enriched bacterial cultures, was consistent with the mineralogy of Rio Tinto cobble coatings characterised by Fernandez-Remolar et al. (2005). Therefore, the enriched iron-oxidising bacterial consortium

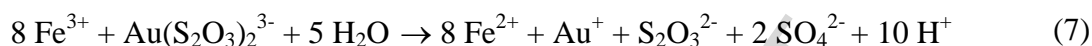
represents a laboratory models of the biogeochemical conditions occurring in an ARD environment.

The increase in Eh of the enriched bacterial cultures, after three weeks of incubation, indicated that the iron-oxidising bacterial consortium was capable of changing the redox potential of their surrounding aqueous environment. Since the spent media-gold system removed 99.5% gold from solution and the fresh media-gold system had no reaction, the spent media must have had excess ferric iron to destabilise the gold thiosulfate (Reaction 7).

The experimental-gold systems represented different modes in which iron-oxidising bacteria could indirectly and directly affect the stability of gold (I) thiosulphate in ARD environments. It is important to note that under acidic and oxidised conditions, the reduction rate of solid ferric iron, e.g. jarosite, is slow in comparison to aqueous ferric iron, e.g., spent media, (Brown, 1971). Furthermore, thiosulphate can remain stable in solution across a wide range of pH when the ligand is complexed with gold (Lengke et al., 2005); however, the gold (I) thiosulphate complex is no longer stable under acidic conditions when ferric iron is present (Alymore and Muir, 2001). In this study, gold (I) thiosulphate stability was demonstrated by the fresh media-gold system and the chemical controls that did not remove gold from solution over 24 hours (Table 1). The indirect destabilisation of gold (I) thiosulphate by iron-oxidising bacteria was demonstrated by the entire enriched bacterial culture-, spent media- and jarosite-gold systems. In these reaction systems, the presence of bacterially-mediated ferric iron was an important oxidant that caused to the initial destabilisation of the gold (I) thiosulphate complex which could have "liberated" ionic gold (Reaction 7). Once the gold (I) thiosulphate

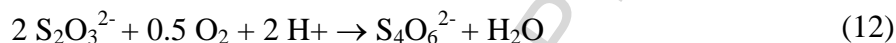
complex was destabilised, disproportionation of residual thiosulphate intuitively occurred forming hydrogen sulphide (Reaction 8) that contributed to the reduction of ionic gold as gold sulphide (Reaction 9). Of these three experimental-gold systems, jarosite removed the least amount of gold from solution. However, the amount of gold removed from the spent media- and stationary phase bacterial culture-gold system was similar (Table 1). This suggested that aqueous ferric iron was the primary factor responsible for the destabilisation and subsequent reduction of gold (I) thiosulphate in the bacterial culture-gold system (Fig. 3b). Morris et al. (2002) demonstrated that colloidal gold sulphide begins by rapid nucleation of $Au_nS_m^-$ and that varying colloidal sizes can occur depending on the presence of excess sulphur. The rapid precipitation of colloidal gold sulphide and formation of colloidal gold sulphide clusters in the spent media-gold system (Fig. 4) was consistent with the studies by Morris et al., (2002). More importantly these results indicated that the rate of aqueous ferric iron reduction, during gold (I) thiosulphate destabilisation, was faster relative to solid ferric iron from the jarosite-gold system (Fig. 5). It should be noted, however, that ionic gold could have been reduced via oxidation of previously reduced iron (Reaction 10) indicating that the cascade of chemical reactions could potentially be a cyclical process (Reaction 7-10). Although colloidal gold was not observed in bacterial culture-, spent media- and jarosite-gold systems, the possibility of gold reduction by ferrous iron should not be negated in natural systems. XANES analysis of the bacterial culture-, spent media-, and jarosite-gold systems indicated that a gold thio-organic compound, e.g., thiomalate, did not form or if it did, was a highly unstable transient species. Immobilisation of gold (I) thiosulphate in these three

experimental-gold systems occurred as reduce gold sulphide and was likely attributed to the relative abundance of sulphur in these systems.



Zusuki (1999) described two possible enzymatic pathways that involve either cleaving or oxidation of thiosulphate ligands. Iron-oxidising bacteria metabolically oxidise ferrous iron to obtain energy; however, these acidophilic chemolithotrophs also use either S transferase to cleave or thiosulphate-oxidising enzymes to oxidise thiosulphate ligands as an alternate means to obtain energy (Singer and Stumm, 1970; Zusuki, 1999). Studies by Lengke and Southam (2005) demonstrated that the reduction of gold (I) thiosulphate and precipitation of colloidal gold occurred along cellular membranes of *Acidithiobacillus thiooxidans* and highlighted the importance of thiosulphate ligand oxidation via bacterial metabolic activity. In this study, enzymatic thiosulphate cleaving (Reaction 11) or oxidation (Reaction 12) of gold (I) thiosulphate could have occurred within the cellular membrane where electron transport processes take place. This initial gold (I) thiosulphate destabilisation subsequently would have enabled the liberation of ionic gold. Extensive extracellular mineralisation of iron-oxidising bacteria in bacterial-gold systems indicated that nanometre-size gold colloids formed on the extracellular surface of bacterial cells. Organic material derived from bacteria and extracellular polymeric substances acted as sources of electron donors that directly reduced ionic gold to elemental gold (Reaction 13). Bacterial gold-

mineralisation resulted in cell wall disruption leaving no evidence of the semi-permeable membrane other than relic cell envelope structures (Fig. 6b, c).



The chemical reaction of gold (I) thiosulphate destabilisation and reduction, described above, explain the quantitative and qualitative observations of gold immobilisation from each experimental-gold system. More importantly, these reactions demonstrate the indirect and direct affects of iron-oxidising bacteria on the stability of gold (I) thiosulphate under acidic, oxidised, iron-rich conditions. The weathering of gold-bearing, poly-metallic sulphides by chemolithoautotrophic bacteria contributes in part to the formation and maintenance of ARD environmental conditions. Under these biogeochemical conditions gold can be solubilised as a gold (I) thiosulphate complex (Puddephatt, 1978). However, as demonstrated by these laboratory experiments, both aqueous ferric iron and jarosite, i.e., an important intermediate component of gossan formation, contribute to the destabilisation of gold (I) thiosulphate resulting in the formation of colloidal gold sulphide. Furthermore, bacteria directly reduce this complex as gold colloids. It is reasonable to suggest that the environments in which chemolithoautotrophic bacteria contribute to the dissolution of gold-bearing poly-metallic sulphides complete the cycling of gold by also contributing to gold precipitation. Therefore in natural ARD environments where weathering of gold-bearing sulphides is occurring, the presence of iron-oxidising bacteria can contribute to secondary gold enrichment.

5. Conclusion

In this study, enriched cultures of an acidophilic iron-oxidising bacterial consortium had an indirect effect on the stability of gold (I) thiosulphate by increasing ferric iron concentrations via ferrous iron oxidation. Upon the destabilisation of the gold complex, systems bearing ferric iron produced colloidal gold sulphide of varying sizes whereas the bacterial-gold system produced colloidal gold. These experimental systems were laboratory models demonstrating the accelerated biogeochemical reduction of gold (I) thiosulphate within near-surface weathering environments dominated by acidic oxidising conditions. Therefore, it can be interpreted that the mobility of gold as a soluble gold (I) thiosulphate complex in an acidic, oxidised weathering environment is unlikely. However, these bacterial conditions, influenced by active bacterial metabolism, promote gold reduction and the potential for mobility as colloidal gold sulphide. From a geochemical perspective, the destabilisation, reduction and biomineralisation of gold (I) thiosulphate would occur concurrently with the continued oxidation and weathering of the host metal sulphides. As a result, this biogeochemical cycling of gold could lead to supergene gold enrichment.

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Figure Captions

Figure 1. A field photograph of Tinto River, Spain. Boulder-size rocks were coated in a range of hydrated (orange-yellow) to dehydrated (red) iron oxyhydroxide mineral precipitates. A biofilm containing iron oxyhydroxide, submersed in the water (circle), was sampled and was the source material for iron-oxidising bacterial cultures (inset).

Figure 2. A powdered X-ray diffractogram of the iron oxyhydroxide precipitate from the bacterial culture that was found to consist of ammoniojarosite and hydroniumjarosite.

Figure 3. A high-resolution SEM micrograph of an enriched iron-oxidising bacterial culture prior to exposure to gold (I) thiosulphate. Bacteria were rod-shaped and associated with jarosite (a). A high-resolution SEM micrograph of the bacterial culture-gold system exposed to 20 mM gold for 24 hours. Individual rod-shaped cells and clusters of 100 nm size gold sulphide colloids occurred on the surface of the jarosite (b and EDS inset).

Figure 4. A high-resolution secondary electron-SEM micrograph of a spent media-gold system exposed to 20 mM gold for 24 hours. The black precipitate was found to consist of clusters of colloidal gold sulphide ranging in size from 200 nm to 2 μ m in diameter.

Figure 5. An ultra-thin section TEM micrograph demonstrating jarosite as an acicular, radial structures approximately 100 nm in diameter (a). A jarosite-gold system exposed to 20 mM gold for 24 hours demonstrated that acicular jarosite “filaments” dissolved and

the remaining minerals incorporated 10 nm sized gold sulphide colloids (b). Note the C peak is attributed to the Epon embedding plastic and the unlabelled peak is Cu from the TEM grid.

Figure 6. TEM-EDS characterisation of a bacterial-gold system. Ultra thin section TEM micrograph of a bacterial cell exposed to 20 mM gold for 24 hours (a). Gold immobilisation occurred as extensive extracellular precipitation of gold colloids that were 3 nm in size and appeared to be associated with remnant iron oxyhydroxides or perhaps lipopolysaccharides or exopolymer at the cell surface (b).

Figure 7. XANES spectra of the entire bacterial culture-, spent media, jarosite-gold systems and the four reference standards. The gold compounds have characteristic peaks occurring after the L-III absorption edge. Gold sulphide formed in all three of these experimental-gold systems when exposed to 20 mM gold for 1 hour.

Table Caption

Table 1. Chemical analysis of experimental-gold system components.

Amount of Au Immobilised from Solution (mM), pH and Eh (V) of Experimental-Gold Systems over Time (hours)

Stationary phase bacterial culture-gold system

Time	Au ^a	pH	Eh	Au ^b	pH	Eh	Au ^c	pH	Eh	Au ^d	pH	Eh	Au ^e	pH	Eh
1	0.0016	2.2	0.6	0.018	2.2	0.6	0.19	2.2	0.6	2.01	2.2	0.6	19.87	2.2	0.55
		4	2		4	2		5	2		3	1		3	
2	0.0016	2.2	0.6	0.018	2.2	0.6	0.20	2.2	0.6	2.03	2.2	0.5	20.07	2.2	0.55
		4	2		4	1		5	0		5	9		5	
4	0.0016	2.2	0.6	0.019	2.2	0.6	0.20	2.2	0.5	2.03	2.2	0.5	20.15	2.2	0.55
		3	1		5	0		7	8		5	6		6	
8	0.0017	2.2	0.6	0.019	2.2	0.6	0.20	2.2	0.5	2.03	2.2	0.5	20.19	2.2	0.51
		5	1		8	0		7	8		6	6		8	
24	0.0018	2.2	0.6	0.019	2.2	0.5	0.20	2.2	0.5	2.03	2.2	0.5	20.28	2.2	0.51
		4	1		8	9		9	8		6	3		8	

Spent media-gold system

Time	Au ^e	pH	Eh
24	20.21	2.2	--
		8	

Fresh media-gold system

Time	Au ^e	pH	Eh
24	0	2.3	--
		0	

Jarosite-gold system

Time	Au ^e	pH	Eh
24	6.25	1.3	--
		2	

Bacterial-gold system**Time**
24**Au^e**
0.89
pH
1.3
Eh
--
0

Gold stock added experimental-gold systems: ^a0.0019 mM Au, ^b0.022 mM Au, ^c0.21 mM Au, ^d2.05 mM Au, ^e20.3 mM Au^e



Figure 1

ACCEPTED

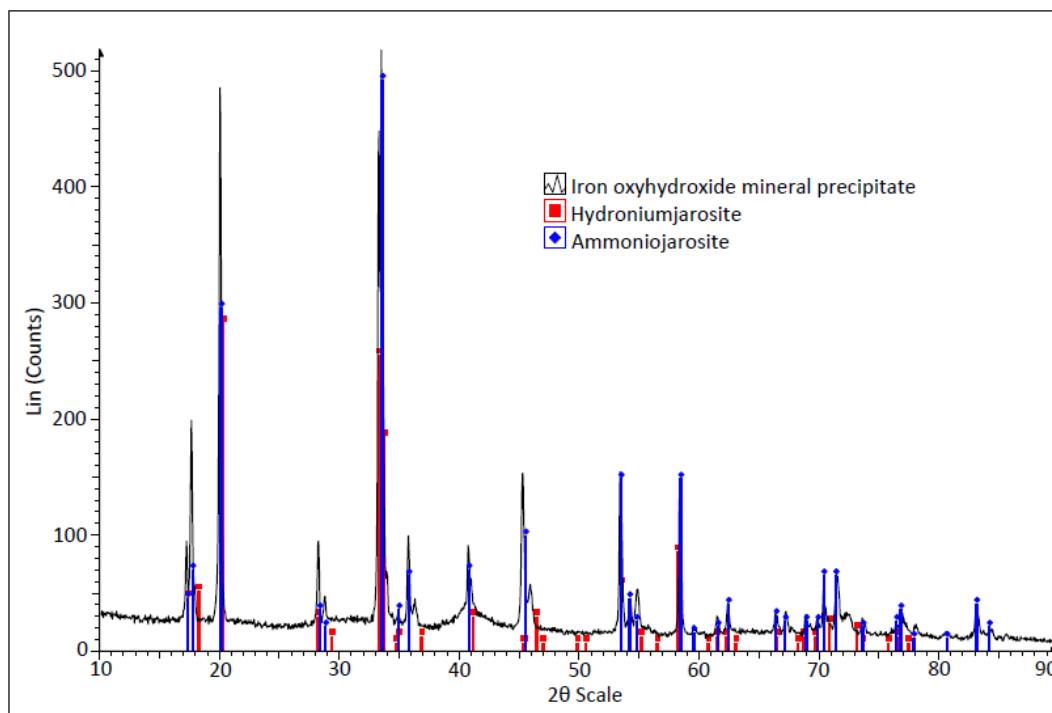


Figure 2

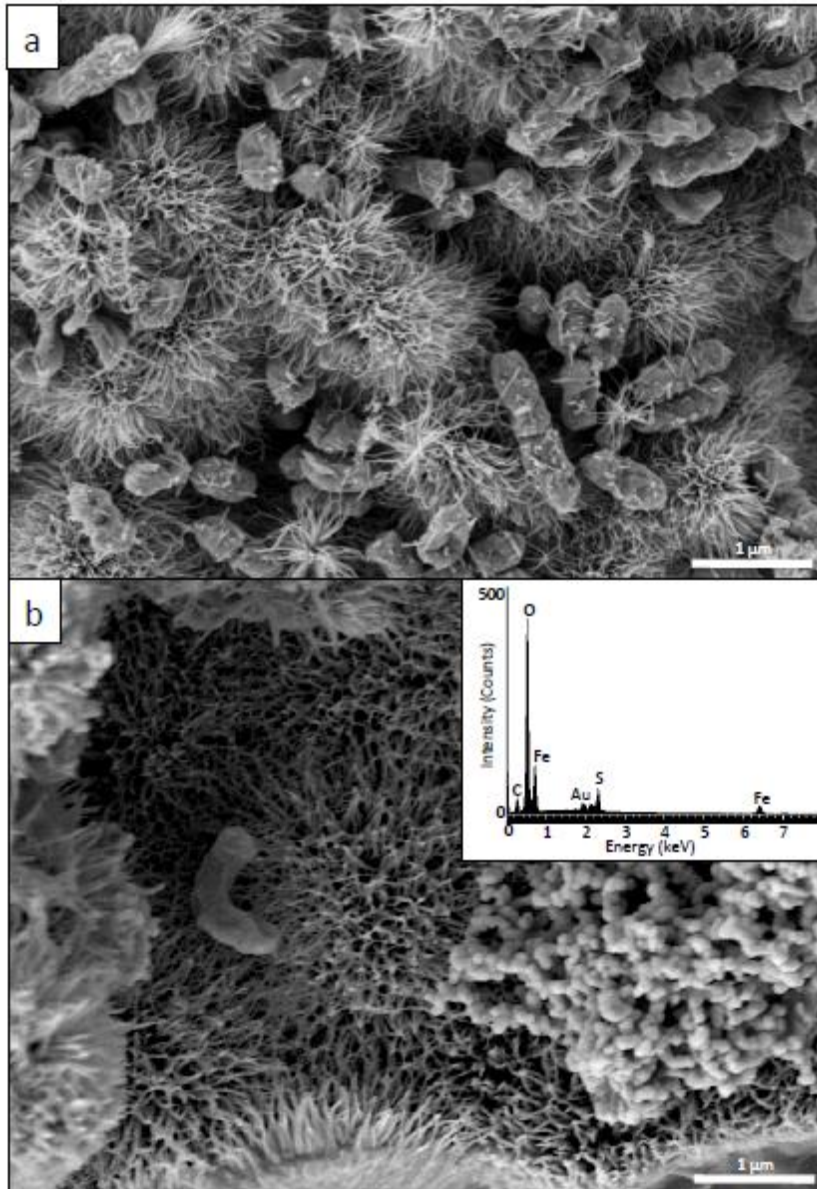


Figure 3

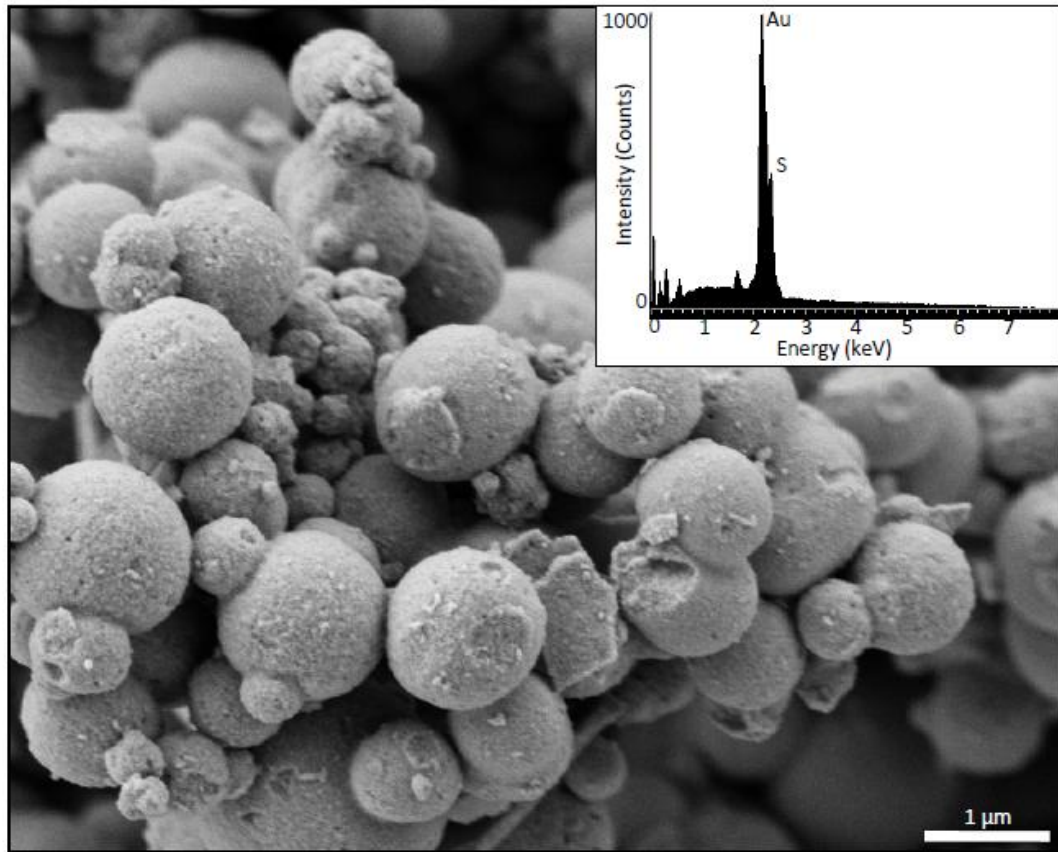


Figure 4

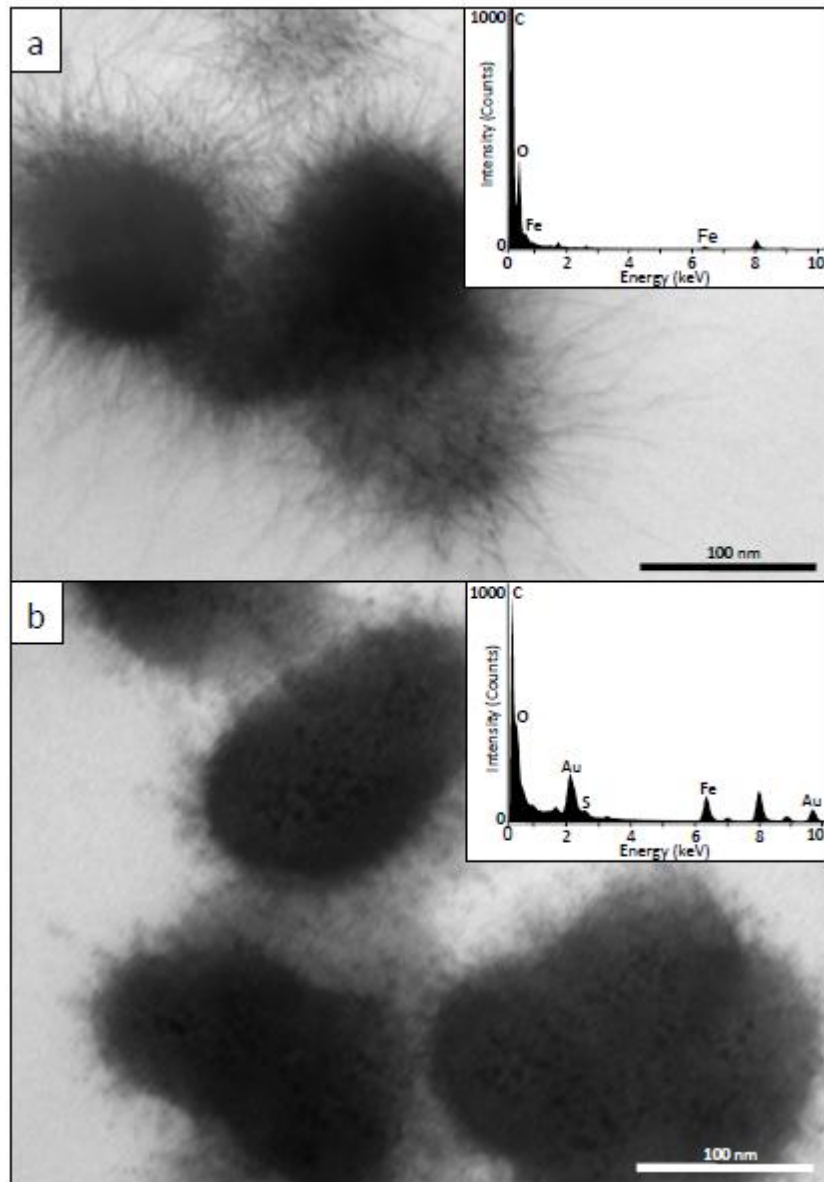


Figure 5

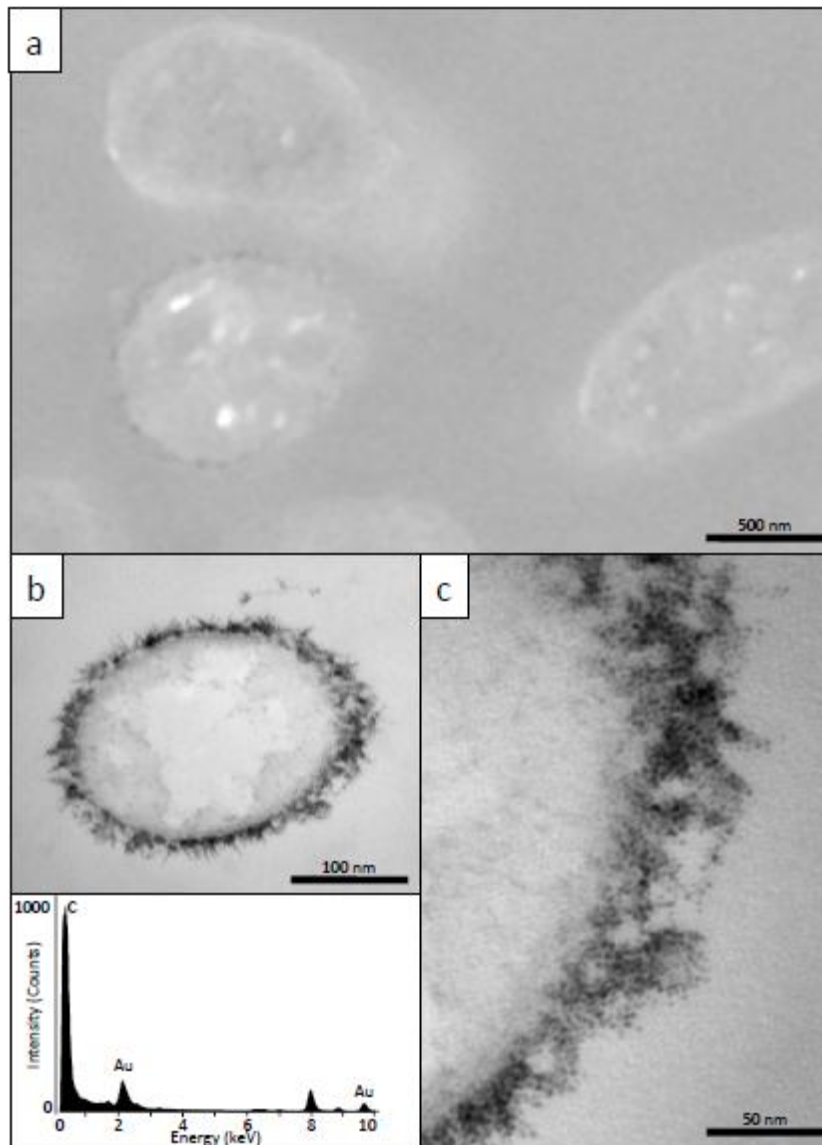


Figure 6

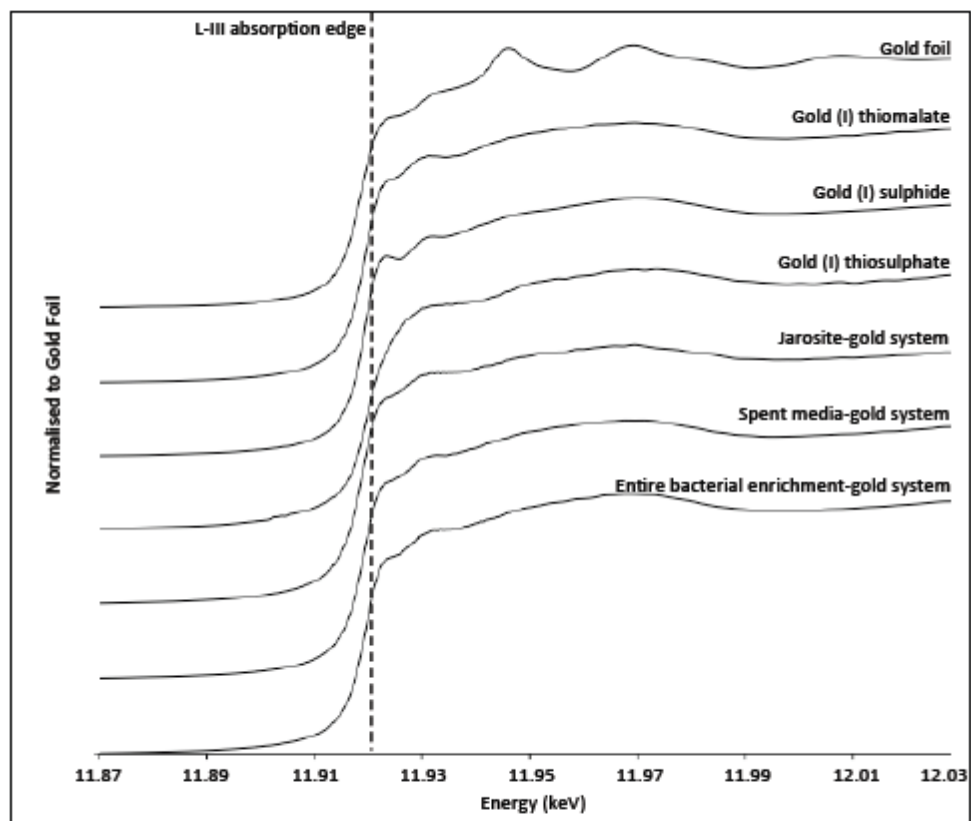


Figure 7

Highlights

- Gold (I) thiosulphate is unstable in the presence of iron-oxidising bacteria.
- Gold (I) thiosulphate is unstable in the presence of ferric iron.
- Gold (I) thiosulphate is transformed into gold sulphide or elemental gold.
- Precipitate of gold in jarosite can be preserved in gossans over geological time.