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Bioaccumulation of gold by sulfate-reducing bacteria cultured in the presence of gold(I)-thiosulfate complex

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Abstract

A sulfate-reducing bacterial (SRB) enrichment, from the Driefontein Consolidated Gold Mine, Witwatersrand Basin, Republic of South Africa, was able to destabilize gold(I)-thiosulfate complex ($Au(S_2O_3)_2^{3-}$) and precipitate elemental gold. The precipitation of gold was observed in the presence of active (live) SRB due to the formation and release of hydrogen sulfide as an end-product of metabolism, and occurred by three possible mechanisms involving iron sulfide, localized reducing conditions, and metabolism. The presence of biogenic iron sulfide caused significant removal of gold from solutions by adsorption and reduction processes on the iron sulfide surfaces. The presence of gold nanoparticles within and immediately surrounding the bacterial cell envelope highlights the presence of localized reducing conditions produced by the bacterial electron transport chain via energy generating reactions within the cell. Specifically, the decrease in redox conditions caused by the release of hydrogen sulfide from the bacterial cells destabilized the $Au(S_2O_3)_2^{3-}$ solutions. The presence of gold as nanoparticles (<10 nm) inside a sub-population of SRB suggests that the reduction of gold was a part of metabolic process. In late stationary phase or death phase, gold nanoparticles that were initially precipitated inside the bacterial cells, were released from the cells and deposited in the bulk solution as addition of gold nanoparticles that already precipitated in the solution. Ultimately, the formation of micrometer-scale sub-octahedral and octahedral gold and spherical aggregates containing octahedral gold was observed.

1. Introduction

The accumulation and migration of gold in natural systems has long been the subject of intense debate. Gold is known to form complexes with chloride, $AuCl_2^-$ or $AuCl_4^-$, (Webster, 1986; Benedetti and Boulegue, 1991; Ran et al., 2002) thiosulfate ($Au(S_2O_3)_2^{3-}$) (Goleva et al., 1970; Plyusnin et al., 1981; Mann, 1984; Webster, 1986; Benedetti and Boulegue, 1991), bisulfide and sulfide ($Au(HS)_2^-, AuS^-, Au_2(HS)_2S^{2-}$) (Krauskopf, 1951; Seward, 1973; Webster, 1986), and possibly sulfite ($Au(SO_3)_2^{3-}$) (Pitul'ko, 1976). At circumneutral to alkaline pH values, thiosulfate is a product of chemical oxidation of reduced sulfur compounds (Goldhaber, 1983), and is an intermediate sulfur species produced by microbiological reactions (Suzuki, 1999). Since it is also stable in a geochemical field encompassing mildly acid to highly alkaline pH, and moderately oxidizing to reducing conditions (Mineyev, 1976; Goldhaber, 1983; Webster, 1986), it is considered one of the most likely or important gold complexing agents in natural systems (Saunders, 1989; Vlassopoulos and Wood, 1990).

Bioaccumulation of gold by bacteria has been described in a wide range of natural environments, from Australia to Venezuela to Alaska (Bischoff et al., 1992; Watterson, 1992; Bischoff, 1994, 1997). Several laboratory experiments have investigated the interaction of bacteria with gold using the gold(III)-chloride ($AuCl_4^-$) complex (e.g., Southam and Beveridge, 1994, 1996; Kashefi et al., 2001; Karthikeyan and Beveridge, 2002; Nakajima, 2003), however, only one recent study has described the bioaccumulation of gold from gold(I)-thiosulfate complex ($Au(S_2O_3)_2^{3-}$)

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(Lengke and Southam, 2005). This previous study of bioaccumulation of gold by thiosulfate oxidizing bacteria demonstrated that the precipitation of gold from $Au(S_2O_3)_2^{3-}$ occurred inside the bacterial cells and was a part of metabolic process once uncomplexed thiosulfate was consumed. When gold nanoparticles inside the cells were released through the cell envelope to the bulk solution, extracellular gold minerals, as micrometer-scale networks of wire gold and octahedral gold, were ultimately formed.

Because of the importance of gold(I)-thiosulfate complex ($Au(S_2O_3)_2^{3-}$) in natural systems, an understanding of bioaccumulation of gold from this complex is important. Thus, the goal of this study was to investigate the interaction between gold(I)-thiosulfate complex ($Au(S_2O_3)_2^{3-}$) and a bacterial enrichment that is dominated by sulfate-reducing bacteria (SRB). The formation of gold from the interaction of SRB with $Au(S_2O_3)_2^{3-}$ has not been investigated. In this study, thiosulfate was used as the terminal electron acceptor instead of sulfate. The enrichment culture of SRB was chosen in this study because these bacteria are broadly distributed on earth and commonly found in mining areas.

Sulfate-reducing bacteria (SRB) typically oxidize organic compounds using sulfate as their terminal electron acceptor. Hydrogen sulfide (H_2S), produced as one of the major end-products of their metabolism, has a strong affinity for metals and readily forms insoluble compounds by the following reactions (Trudinger et al., 1985):

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^- \quad (1)$$

$$M^{2+} + H_2S \rightarrow MS_{(s)} + 2H^+ \quad (2)$$

Sulfate-reducing bacteria (SRB) are ecologically diverse and tend to be enriched wherever sulfoxyanions, e.g., sulfate or thiosulfate, are present along with a sufficient supply of organic matter to create anaerobic conditions (Trudinger et al., 1985; Peck, 1992; Kleikemper et al., 2002).

2. Materials and methods

2.1. Chemicals

Aqueous $Au(S_2O_3)_2^{3-}$ used in this study were prepared from $Na_3Au(S_2O_3)_2{\cdot}2H_2O$ (Alfa Aesar Company, Ward Hill, Massachusetts, USA), dissolved in distilled, deionized water (DDI) at 18.2 $M\Omega cm^{-1}$ obtained from a Millipore system. The $Au(S_2O_3)_2^{3-}$ solutions were filter-sterilized using a 0.45-μm membrane filtration before being added to both bacterial and abiotic experiments.

2.2. Bacterial enrichment and enumeration

Heterotrophic SRB enrichments were obtained from a water sample collected from a borehole, 3.2 km below land surface in the Driefontein Consolidated Gold Mine, Witwatersrand Basin, South Africa, in 1998. A primary enrichment in the form of a mixed culture that included representatives of SRB was grown by inoculating 1 mL of the water sample in sealed borosilicate serum vials containing 10 mL of SRB medium (total volume of 11 mL), modified from Fortin et al. (1994) (Table 1). This enrichment culture was grown at room temperature (RT; 23–25 °C) for two weeks. Subsequent secondary and tertiary enrichments were prepared by inoculating 1 mL ($\sim 10^7$ bacteria) of the previous enrichment into 11 mL of fresh SRB medium in filled, sealed borosilicate serum vials (total volume of 12 mL) and incubated as above. From the tertiary enrichment, the SRB enrichment was then cultured in filled, sealed borosilicate serum bottles containing thiosulfate growth medium, with and without the addition of iron (Table 1). Positive SRB enrichments, indicated by a black precipitate of iron sulfide (in a medium containing iron) or a turbid solution (in a medium without the presence of iron), were transferred (8% [vol./vol.] of inoculum) three times before use as inoculum in the experiments. The number of viable SRB was determined by the most-probable-number (MPN) technique (as described in Section 2.5).

Before the SRB culture was used in the experiments, populations of facultative aerobes and anaerobic bacteria including SRB were determined on agar plates of SRB medium. The agar plates of SRB medium were used because the culture was grown in similar medium. The results of bacterial enumeration show that the bacterial enrichment was dominated by SRB; aerobes and facultative anaerobes were not detected.

2.3. Bacterial experiments

Three types of bacterial experiments were conducted to examine the role of SRB on the accumulation of gold from $Au(S_2O_3)_2^{3-}$: Type 1, in which $Au(S_2O_3)_2^{3-}$ was added to the cultures after they reached a stationary phase; Type 2, in which bacteria were grown in thiosulfate growth medium containing $Au(S_2O_3)_2^{3-}$; and Type 3, in which $Au(S_2O_3)_2^{3-}$ was added to dead, autoclaved cultures. Type 1 bacterial experiments were conducted to model conditions in which the presence of an active population of SRB receives an influx of $Au(S_2O_3)_2^{3-}$; Type 2 experiments were conducted to determine whether these bacteria can grow in the presence of $Au(S_2O_3)_2^{3-}$ and further precipitate gold from solutions; and Type 3 bacterial experiments were conducted to determine whether the presence of organic matter released by the bacteria through excretion or autolysis can promote the precipitation of gold from $Au(S_2O_3)_2^{3-}$. In order to accelerate the rate of $Au(S_2O_3)_2^{3-}$ reduction in our experiments, we increased the concentrations of gold and organics several orders of magnitude higher than those found in nature. To assess the effect of iron sulfide on gold reduction, Types 1 and 2 bacterial experiments were conducted in the presence and absence of iron (using iron acetate) to compare systems containing iron sulfide with the systems without iron sulfide on gold reduction.

Table 1
Media compositions used in the bacterial and abiotic experiments

Constituent	SRB medium	Physiological saline	SRB medium (agar late)	Thiosulfate medium			
				Without iron (Types 2a, 1b, 2b, and 3)	Without iron (Type 1a)	With iron (Types 2a, 1b, 2b, and 3)	With iron (Type 1a)
Tryptone	10.0		10.0	10.0	10.0	10.0	10.0
Yeast extract	1.0		1.0	1.0	1.0	1.0	1.0
Iron sulfate ($FeSO_4 \cdot 7H_2O$)	0.5		0.5				
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	2.0		2.0				
L-Ascorbic acid	1.5	3.75	1.5				
Sodium lactate (60%)	6 ml/L		6 ml/L	6 ml/L	6 ml/L	6 ml/L	6 ml/L
Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)				1.23		1.23	
Iron acetate ($Fe(C_2H_3O_2)_2$)						0.269	0.269
Sodium chloride (NaCl)		8.5					
Agar			15.0				
Sodium sulfide ($Na_2S \cdot 9H_2O$)					2.46		2.46

In the experimental code, the letter (a or b) represents abiotic or bacterial experiments, and the number is the experimental type, i.e., Types 1, 2, and 3. Unless noted, all values are given in g/L and mixed in distilled, deonized water. With the exception of the SRB medium in agar plate, all media are filtered sterilized using 0.45 μm membrane filtration, and the medium pH is adjusted to about 7.4 using NaOH.

2.3.1. Type 1 bacterial experiment

Sulfate-reducing bacteria (SRB) enrichment was grown at RT in sealed borosilicate serum bottles containing 11 mL of thiosulfate growth medium (Table 1) into late stationary phase (about 2–3 weeks). Before the addition of $Au(S_2O_3)_2^{3-}$ solutions, iron sulfide or sulfide was present, and sulfide concentrations were 5.63 and 6.56 mM in the systems containing iron and without iron, respectively. One milliliter of $Au(S_2O_3)_2^{3-}$ (final gold concentrations of 0, 0.22, 0.53, and 2.55 mM) was injected into the serum bottles (total volume of 12 mL). The experiments were conducted for 53 days after the addition of $Au(S_2O_3)_2^{3-}$; pH, Eh, total gold and iron (if present), sulfide, and the SRB population were measured with time.

2.3.2. Type 2 bacterial experiment

To initiate the experiments, 1 mL of gold solution (final gold concentrations of 0, 0.01, 0.02, 0.05, 0.07, 0.14, 0.22, and 0.35 mM) was added to 10 mL of thiosulfate growth medium prior to inoculating SRB culture. The medium contained in sealed borosilicate serum bottles was then inoculated with 1 mL of SRB culture (8% [vol./vol.] inoculum) containing $\sim 10^7$ cells/mL (total volume of 12 mL). The inoculated bottles were incubated at RT for 6 days; pH, Eh, total gold and iron (if present), sulfide, and the SRB population were measured with time.

2.3.3. Type 3 bacterial experiment

The procedures used in Type 3 bacterial experiments were similar to those in Type 1 bacterial experiments except that the SRB culture was killed by autoclaving for 1 h at 121 °C after growth of the SRB culture to stationary phase. One milliliter of $Au(S_2O_3)_2^{3-}$ solution (final gold concentrations of 0, 0.24, and 1.14 mM) was added to 11 mL of dead, autoclaved SRB at RT (total volume of 12 mL).

All bacterial experiments were conducted in duplicate, in the dark. Six to eleven serum bottles were used for each bacterial experiment Type. The solution from each serum bottle was used for the measurements of pH, Eh, total gold and iron (if present), sulfide, and the SRB population in each sampling time. This procedure was conducted to collect enough solution for the indicated measurements and to avoid contamination. All samples were collected in anaerobic conditions under nitrogen gas.

2.4. Abiotic experiments

Two types of abiotic experiments were conducted as a chemical control for corresponding bacterial experiments: Type 1, in which $Au(S_2O_3)_2^{3-}$ was added to the thiosulfate growth medium containing sulfide or iron sulfide; and Type 2, in which $Au(S_2O_3)_2^{3-}$ was added to the thiosulfate growth medium (Table 1). The abiotic experiments were conducted without the presence of SRB.

Type 1 abiotic experiments (final gold concentrations of 0, 0.22, 0.53, and 2.55 mM) were conducted in the presence of iron sulfide or sulfide by adding sodium sulfide into the thiosulfate medium (Table 1). In Type 1 bacterial experiments, the SRB culture grown in the presence and absence of iron formed iron sulfide and sulfide, respectively, therefore, iron sulfide or sulfide was added to the medium in the corresponding abiotic experiments. Type 1 abiotic experiments were conducted for 53 days at RT and pH of ~7.7. The solutions in Type 1 abiotic experiments were prepared with sparging nitrogen gas to avoid sulfide oxidation. The Type 2 abiotic experiments were conducted (final gold concentrations of 0, 0.01, 0.02, 0.05, 0.07, 0.14, 0.22, and 0.35 mM) for 14 days at RT and pH ~7.4. All experiments were duplicated and conducted in the dark; pH, Eh, total gold and iron (if present), and sulfide were measured with time.

2.5. Bacterial viability

The effect of $Au(S_2O_3)_2^{3-}$ on SRB viability was monitored during the course of the bacterial experiments using the most-probable-number (MPN) technique. The number of viable SRB was determined using five replicate tenfold dilution with SRB medium (Table 1). The culture was diluted to 10^{-10} in physiological saline (Table 1). One milliliter aliquots of each dilution were transferred to SRB culture tubes containing 7 mL of SRB growth media, and the tubes were incubated at RT for 4 weeks. The presence of SRB in the MPN dilution tubes was evaluated by the formation of a black precipitate of iron sulfide as a positive indication of the presence of SRB. The statistical method of Cochran (1950) was used to calculate MPN values.

2.6. Analyses

The pH and Eh were monitored using a Denver Instrument Basic pH/ORP/temperature-meter. The pH electrode was calibrated using buffer solutions 4, 7, and 10 with analytical uncertainties in measurements of pH of ±0.05 pH unit. The Eh was measured using an ORP electrode and calibrated using ZoBell's solution (Nordstrom, 1977).

Total gold and iron concentrations were measured over the course of the experiments with a Perkin-Elmer 3300-DV Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) instrument. The solutions were filtered through 0.1 μm Millipore membrane filters prior to ICP analyses. The uncertainty in measured gold and iron is ⩽5%, with detection limits of 0.25 μM and 0.018 mM for gold and iron, respectively. Sulfide concentrations were determined by methylene blue method with a Hach DR/890 Colorimeter and a Shimadzu UV–Vis 1200 series spectrometer.

2.7. Transmission electron microscopy (TEM), scanning electron microscopy (SEM), and X-ray diffraction (XRD)

Unstained whole mounts and ultrathin sections of SRB and gold particles from the experimental products were examined with a Phillips CM-10 transmission electron microscope (TEM) operating at 80 kV and a Phillips EM400T transmission electron microscope (TEM) with energy dispersive X-ray spectrometer (EDS). The whole mounts were prepared by floating Formvar carbon-coated 200 mesh copper grids on a drop of culture for several minutes to allow the bacteria and any fine-grained minerals to attach to the grid. The mounts were then washed with DDI water to remove salts from the culture medium and allowed to air dry. Selected area electron diffraction (SAED) patterns of the precipitated solids were obtained by TEM from unstained whole mounts to ascertain the crystallinity of these solids (Southam and Beveridge, 1996).

Samples for ultrathin sections were fixed in 2% glutaraldehyde, enrobed in noble agar, dehydrated in a $25\%_{(aq)}$, $50\%_{(aq)}$, $75\%_{(aq)}$, and $100\%_{(aq)}$ ethanol series and embedded in LR (London Resin) White resin to prevent oxidation of the iron sulfides (Fortin et al., 1994). The embedded samples were ultrathin sectioned on a Reichert-Jung Ultracut E ultramicrotome using a diamond knife to a thickness of 70 nm and collected on Formvar-carbon coated 200 mesh copper grids.

The precipitated solids at the completion of experiments were also collected on 0.1 μm filters, air dried and examined for morphology and composition using a Hitachi S-4500 scanning electron microscope equipped with an energy dispersive spectrometer (SEM/EDS). Bacterial samples were fixed in 2% glutaraldehyde, dehydrated in a $25\%_{(aq)}$, $50\%_{(aq)}$, $75\%_{(aq)}$, and $100\%_{(aq)}$ ethanol series, and critical-point dried to preserve bacterial structure. All samples for SEM/EDS were carbon coated prior to analysis.

The X-ray diffraction (XRD) data were collected from the precipitated solids to ascertain crystallinity. The XRD data was collected at 40 kV and 40 mA in micro diffraction mode with CuKα radiation using a Discover D8 diffractometer (Bruker, Germany) equipped with a General Area Detector System (GADDS).

3. Results

3.1. Bacterial and abiotic experiments

The results of bacterial and abiotic experiments using $Au(S_2O_3)_2^{3-}$ are shown in Figs. 1–6 and Tables 2–4.

3.1.1. Type 1 versus type 3 bacterial experiments

On addition of $Au(S_2O_3)_2^{3-}$ to the SRB cultures (Type 1 bacterial experiments), the soluble gold concentrations decreased significantly with time, while in the presence of

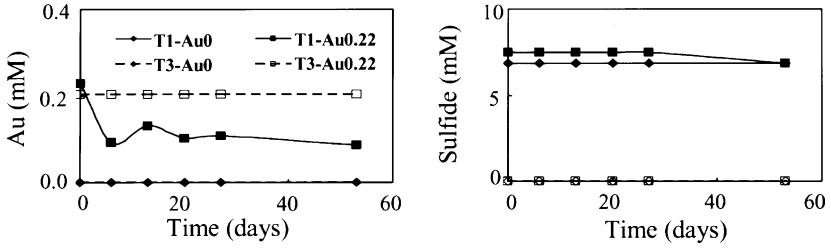

Fig. 1. Representative total soluble gold and sulfide from the Types 1 and 3 bacterial experiments versus time in the systems without iron; for the experimental code, T1 and T3 represent Type 1 and Type 3 bacterial experiments, respectively, and the number is gold concentration in mM.

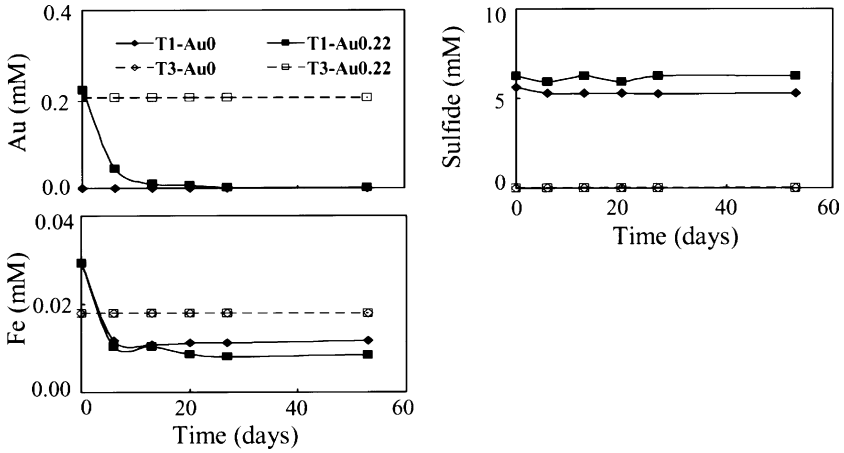

Fig. 2. Representative total soluble gold, iron, and sulfide from the Types 1 and 3 bacterial experiments versus time in the systems containing iron; for the experimental code, T1 and T3 represent Type 1 and Type 3 bacterial experiments, respectively, and the number is gold concentration in mM.

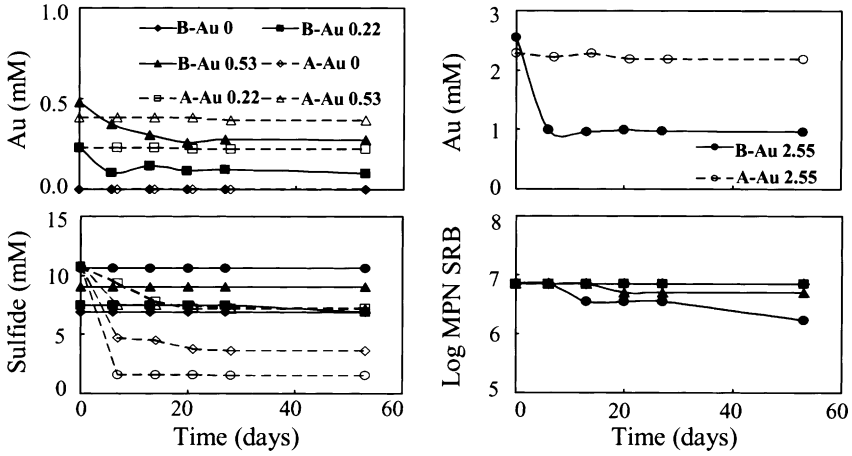

Fig. 3. Representative total soluble gold, sulfide, and the number of viable bacteria from the Type 1 bacterial and abiotic experiments versus time in the systems without iron; for the experimental code, A and B represent abiotic and bacterial experiments, respectively, and the number is gold concentration in mM.

dead, autoclaved SRB (Type 3 bacterial experiments), the soluble gold concentrations were relatively constant in the systems with or without iron (Figs. 1 and 2, Tables 2 and 4). Sulfide concentrations were relatively constant in Type 1 bacterial experiments, while sulfide was not detected in the Type 3 bacterial experiments (Figs. 1 and 2, Tables 2 and 4). In the systems containing iron, total soluble iron decreased with time in Type 1 bacterial experiments, while the concentrations of iron were relatively constant in the Type 3 bacterial experiments (Fig. 1).

3.1.2. Type 1 experiments without iron

After the addition of $Au(S_2O_3)_2^{3-}$ to the stationary phase SRB cultures, the soluble gold decreased with time (Fig. 3). Sulfide concentrations were relatively constant during the experiments (Fig. 3). The viable SRB populations were relatively constant in the systems without gold or with lower gold concentrations ($\leqslant$0.22 mM gold). However, at higher gold concentrations ($\geqslant$0.53 mM gold), SRB populations decreased from 7.0×10^6 to 5.0×10^6 or 1.7×10^6 MPN/mL within 53 days, for 0.53 mM and 2.55 mM gold, respectively (Fig. 3 and Table 2). Eh values decreased slightly from −0.14 to (−0.15)–(−0.19) at gold concentrations of 0, 0.22, and 0.53 mM, but these values slightly increased from −0.3 to −0.2 V at 2.55 mM gold. pH varied between 7.7 and 8.1 during the experiments (Table 2). In the abiotic experiments, total soluble gold concentrations were slightly decreased about 0.01–0.1 mM during the experiments (Fig. 3 and Table 2). Sulfide concentrations decreased significantly from 10.76 to 1.5–7.19 mM (Table 2). Eh and pH were relatively constant during the experiments (Table 2). After 53 days, the gold precipitation in the bacterial systems ranged from 44% to 62%, while in the corresponding abiotic systems, it only ranged about 3–4% (Table 2).

3.1.3. Type 1 experiments containing iron

On addition of $Au(S_2O_3)_2^{3-}$ to the stationary phase SRB cultures, the soluble gold was precipitated from solution within 28 days (Fig. 4). Sulfide concentrations were

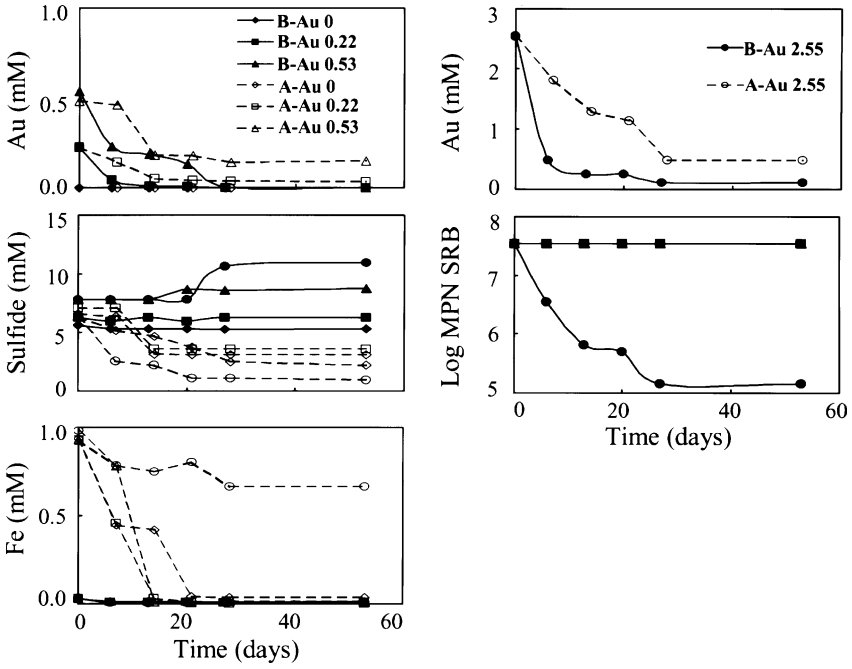

Fig. 4. Representative total soluble gold and iron, sulfide, and the number of viable bacteria from the Type 1 bacterial and abiotic experiments versus time in the systems containing iron; for the experimental code, A and B represent abiotic and bacterial experiments, respectively, and the number is gold concentration in mM.

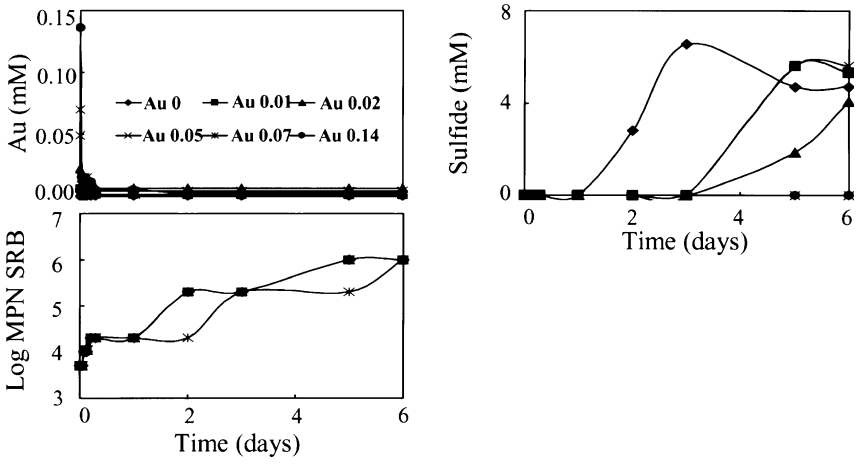

Fig. 5. Representative total soluble gold, sulfide, and the number of viable bacteria from the Type 2 bacterial experiments versus time in the systems without iron; for the experimental code, the number is gold concentration in mM.

relatively constant at ~5.3 and 6.3 mM during the experiments at gold concentrations of 0 and 0.22 mM, respectively, while at higher gold concentrations (0.53 and 2.55 mM gold), these concentrations increased after 21 days (Fig. 4). Total soluble iron concentrations decreased slightly from 0.03 to 0.01 mM (Fig. 4 and Table 2). The initial soluble iron concentrations (at time 0) were low (0.03 mM) due to the formation of iron sulfide. The viable SRB populations were relatively constant in the systems without gold or with lower gold concentrations (⩽0.53 mM gold). However, at higher gold concentrations (2.55 mM gold), SRB populations decreased (Fig. 4 and Table 2). Eh values were relatively constant at (−0.13) to (−0.17) V and slightly increased from −0.3 to −0.21 V at 2.55 mM gold, and pH varied between 7.4 and 7.8 during the experiments (Table 2). In the abiotic experiments, total soluble gold, sulfide, and iron concentrations decreased with time during the experiments (Fig. 4 and Table 2). Eh values generally increased from −0.16 to (−0.11)–(0.00) V, and pH was relatively constant at ~7.7 during the experiments (Table 2). After 53 days, the gold precipitation in the bacterial systems ranged from 96% to 100%, while in the corresponding abiotic systems, it ranged from 69% to 86% (Table 2).

3.1.4. Type 2 experiments without iron

On addition of the SRB inoculum to the thiosulfate growth medium containing $Au(S_2O_3)_2^{3-}$, the soluble gold was precipitated from solution within 1 h (Fig. 5). During

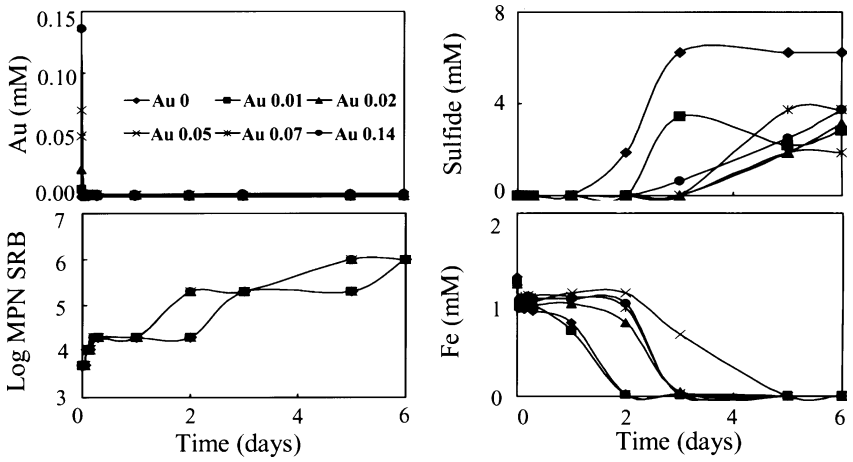

Fig. 6. Representative total soluble gold and iron, sulfide, and the number of viable bacteria from the Type 2 bacterial experiments versus time in the systems containing iron; for the experimental code, the number is gold concentration in mM.

Table 2
Summary of various parameters at the start and at the end of Type 1 bacterial and abiotic experiments performed in the presence and absence of iron

Exp. #	Duration (days)	Initial						Final						
		pH	Eh (V)	Bacteria (MPN/mL)	Total Au (mM)	Total Fe (mM)	Sulfide (mM)	pH	Eh (V)	Bacteria (MPN/mL)	Total Au (mM)	Total Fe (mM)	Sulfide (mM)	Au removal (%)
Bacterial exp.														
Fe-0	53	7.4	−0.14	3.5×10^7	0.00	0.03	5.63	7.4	−0.13	3.5×10^7	0.00	0.01	5.31	—
Fe-0.22	53	7.4	−0.14	3.5×10^7	0.22	0.03	6.25	7.7	−0.16	3.5×10^7	0.00	0.01	6.25	100
Fe-0.53	53	7.4	−0.14	3.5×10^7	0.53	0.03	7.81	7.7	−0.17	3.5×10^7	0.00	0.01	8.75	100
Fe-2.55	53	7.4	−0.30	3.5×10^7	2.55	0.03	7.81	7.8	−0.20	1.4×10^5	0.55	0.01	10.94	96
0	53	7.9	−0.14	7.0×10^6	0.00	—	6.88	7.7	−0.17	7.0×10^6	0.00	—	6.88	—
0.22	53	7.9	−0.14	7.0×10^6	0.23	—	7.50	7.7	−0.19	7.0×10^6	0.09	—	6.88	62
0.53	53	7.9	−0.14	7.0×10^6	0.48	—	9.06	7.7	−0.15	5.0×10^6	0.27	—	9.06	44
2.55	53	7.9	−0.30	7.0×10^6	2.55	—	10.63	8.1	−0.21	1.7×10^6	0.96	—	10.63	62
Abiotic exp.														
Fe-0	53	7.7	−0.16	—	0.00	0.94	6.56	7.7	−0.11	—	0.00	0.04	3.06	—
Fe-0.22	53	7.7	−0.16	—	0.23	0.93	7.03	7.7	−0.12	—	0.03	0.00	3.55	86
Fe-0.53	53	7.7	−0.16	—	0.48	0.93	6.33	7.7	−0.12	—	0.15	0.01	2.19	69
Fe-2.55	53	7.7	−0.16	—	2.54	0.93	6.33	7.7	0.00	—	0.48	0.66	0.94	81
0	53	7.4	−0.17	—	0.00	—	10.76	7.7	−0.11	—	0.00	—	3.59	—
0.22	53	7.7	−0.13	—	0.23	—	10.76	7.7	−0.12	—	0.22	—	7.19	3
0.53	53	7.7	−0.13	—	0.40	—	10.76	7.7	−0.13	—	0.38	—	7.17	4
2.55	53	7.7	−0.13	—	2.28	—	10.76	7.7	−0.13	—	2.18	—	1.50	4

Each of the entries in the column headed Experiment number (Exp. #) indicates whether iron was added (Fe represents the condition with the presence of iron) and the amount of gold was present at the start of the experiment (in mM). The experimental results below are average of duplicate experiments.

the precipitation of gold, sulfide was not detected, and Eh values were about 0.22 V, indicating that reducing conditions were not generated in the bulk solution within this period. Sulfide was detected after 2 or 6 days depending on the initial gold concentration; the higher the gold concentrations, the longer it took to produce measurable sulfide (Fig. 5). In these bacterial systems, the growth of SRB was only observed in the systems containing $\leqslant$0.14 mM gold (Table 3). When growth occurred, the bacterial population increased from 5×10^3 to 10^6 MPN/mL within 6 days (Fig. 5 and Table 3). Eh values decreased from 0.2 to (−0.04)–(−0.18) V, and pH increased slightly from 7.4 to ~7.7 or 7.9. The Eh of the medium was initially positive (0.2 V), but the SRB inoculum was able to grow under this condition, indicating the presence of aerotolerant SRB. In the corresponding abiotic experiments, pH, and total concentrations of gold were relatively constant during the experiments, while Eh values very slightly decreased about 0.06–0.08 V (Table 3). Sulfide was not detected (Table 3).

3.1.5. Type 2 experiments containing iron

On addition of the SRB inoculum to the thiosulfate growth medium containing $Au(S_2O_3)_2^{3-}$, the soluble gold was also precipitated from solutions within 1 h (Fig. 6). During the precipitation of gold, sulfide was not detected, and Eh values were relatively constant ~0.2 V or very slightly decreased about 0.04 V, indicating that the

Table 3
Summary of various parameters at the start and at the end of Type 2 bacterial and abiotic experiments performed in the presence and absence of iron

Exp. #	Duration (days)	Initial						Final						
		pH	Eh (V)	Bacteria (MPN/mL)	Total Au (mM)	Total Fe (mM)	Sulfide (mM)	pH	Eh (V)	Bacteria (MPN/mL)	Total Au (mM)	Total Fe (mM)	Sulfide (mM)	Au removal (%)
Bacterial exp.														
Fe-0	6	7.4	0.20	5.0×10^3	0.00	1.31	0.00	7.9	−0.16	1.0×10^6	0.00	0.01	6.25	—
Fe-0.01	6	7.4	0.20	5.0×10^3	0.01	1.26	0.00	7.9	−0.14	1.0×10^6	0.00	0.01	2.81	98
Fe-0.02	6	7.4	0.20	5.0×10^3	0.02	1.23	0.00	7.9	−0.15	1.0×10^6	0.00	0.00	3.13	99
Fe-0.05	6	7.4	0.20	5.0×10^3	0.05	1.25	0.00	7.9	−0.14	1.0×10^6	0.00	0.00	1.88	99
Fe-0.07	6	7.4	0.20	5.0×10^3	0.07	1.26	0.00	7.9	−0.13	1.0×10^6	0.00	0.00	3.75	99
Fe-0.14	6	7.4	0.20	5.0×10^3	0.14	1.26	0.00	7.9	−0.13	1.0×10^6	0.00	0.00	3.75	98
Fe-0.22[a]	6	7.4	0.20	5.0×10^3	0.22	1.26	0.00	7.4	—	—	—	—	—	—
Fe-0.35[a]	6	7.4	0.20	5.0×10^3	0.35	1.26	0.00	7.4	—	—	—	—	—	—
0	6	7.4	0.29	5.0×10^3	0.00	—	0.00	7.4	−0.18	1.0×10^6	0.00	—	4.69	—
0.01	6	7.4	0.29	5.0×10^3	0.01	—	0.00	7.9	−0.13	1.0×10^6	0.00	—	5.31	83
0.02	6	7.4	0.29	5.0×10^3	0.02	—	0.00	7.7	−0.11	1.0×10^6	0.01	—	4.06	70
0.05	6	7.4[b]	0.29	5.0×10^3	0.05	—	0.00	7.7	−0.09	1.0×10^6	0.00	—	5.63	97
0.07	6	7.4[b]	0.29	5.0×10^3	0.07	—	0.00	7.4	−0.03	1.0×10^6	0.00	—	0.00	94
0.14	6	7.4[b]	0.29	5.0×10^3	0.14	—	0.00	7.4	−0.04	1.0×10^6	0.00	—	0.00	98
0.22[a]	6	7.4[b]	0.29	5.0×10^3	0.22	—	0.00	7.4	—	—	—	—	—	—
0.35[a]	6	7.4[b]	0.29	5.0×10^3	0.35	—	0.00	7.4	—	—	—	—	—	—
Abiotic exp.														
Fe-0	14	7.4	0.20	—	0.00	1.31	—	7.4	0.24	—	0.00	1.23	—	—
Fe-0.01	14	7.4	0.20	—	0.01	1.26	—	7.4	0.22	—	0.01	1.25	—	0
Fe-0.02	14	7.4	0.20	—	0.02	1.23	—	7.4	0.22	—	0.02	1.19	—	0
Fe-0.05	14	7.4	0.20	—	0.05	1.25	—	7.4	0.25	—	0.05	1.24	—	0
Fe-0.07	14	7.4	0.20	—	0.07	1.26	—	7.4	0.21	—	0.07	1.26	—	0
Fe-0.14	14	7.4	0.20	—	0.14	1.26	—	7.4	0.22	—	0.14	1.23	—	0
0	14	7.4	0.29	—	0.00	—	—	7.4	0.23	—	0.00	—	—	—
0.01	14	7.4	0.29	—	0.01	—	—	7.4	0.22	—	0.01	—	—	0
0.02	14	7.4	0.29	—	0.02	—	—	7.4	0.22	—	0.02	—	—	0
0.05	14	7.4	0.29	—	0.05	—	—	7.4	0.21	—	0.05	—	—	0
0.07	14	7.4	0.29	—	0.07	—	—	7.4	0.23	—	0.07	—	—	0
0.14	14	7.4	0.29	—	0.14	—	—	7.4	0.22	—	0.14	—	—	0

Each of the entries in the column headed Experiment number (Exp. #) indicates whether iron was added (Fe represents the condition with the presence of iron) and the amount of gold was present at the start of the experiment (in mM). The experimental results below are average of duplicate experiments.
[a] Did not grow.
[b] Measured with pH indicator strips.

Table 4
Summary of various parameters at the start and at the end of Type 3 bacterial experiments performed in the presence and absence of iron

Exp. #	Duration (days)	Initial						Final						
		pH	Eh (V)	Bacteria (MPN/mL)	Total Au (mM)	Total Fe (mM)	Sulfide (mM)	pH	Eh (V)	Bacteria (MPN/mL)	Total Au (mM)	Total Fe (mM)	Sulfide (mM)	Au removal (%)
Fe-0	53	7.7	0.40	—	0.00	0.02	—	7.7	0.40	—	0.00	0.02	—	—
Fe-0.22	53	7.7	0.40	—	0.20	0.02	—	7.7	0.40	—	0.22	0.02	—	0
Fe-1.14	53	7.7	0.40	—	1.14	0.02	—	7.7	0.40	—	1.13	0.02	—	0
0	53	7.7	0.40	—	0.00	—	—	7.7	0.40	—	0.00	—	—	—
0.22	53	7.7	0.40	—	0.22	—	—	7.7	0.40	—	0.22	—	—	0
1.14	53	7.7	0.40	—	1.14	—	—	7.7	0.40	—	1.14	—	—	0

Each of the entries in the column headed Experiment number (Exp. #) indicates whether iron was added (Fe represents the condition with the presence of iron) and the amount of gold was present at the start of the experiment (in mM). The experimental results below are average of duplicate experiments.

reducing conditions were not formed within this period. Sulfide was first detected after 2 or 3 days and increased in concentration significantly with time (Fig. 6). In these experiments, iron sulfide was precipitated after gold was removed from solution. Total soluble iron concentrations decreased significantly with time (Fig. 6 and Table 3). In these bacterial systems, the growth of SRB was only observed in the systems containing $\leqslant$0.14 mM gold, suggesting that gold was toxic towards SRB culture at higher gold concentrations ($\geqslant$0.22 mM gold) (Table 3). When growth occurred, the bacterial population increased from 5×10^3 to 10^6 MPN/mL within 6 days (Fig. 6 and Table 3). Eh values decreased from 0.2 to (−0.13)–(−0.16) V, and pH increased slightly from 7.4 to ~7.9. In the corresponding abiotic

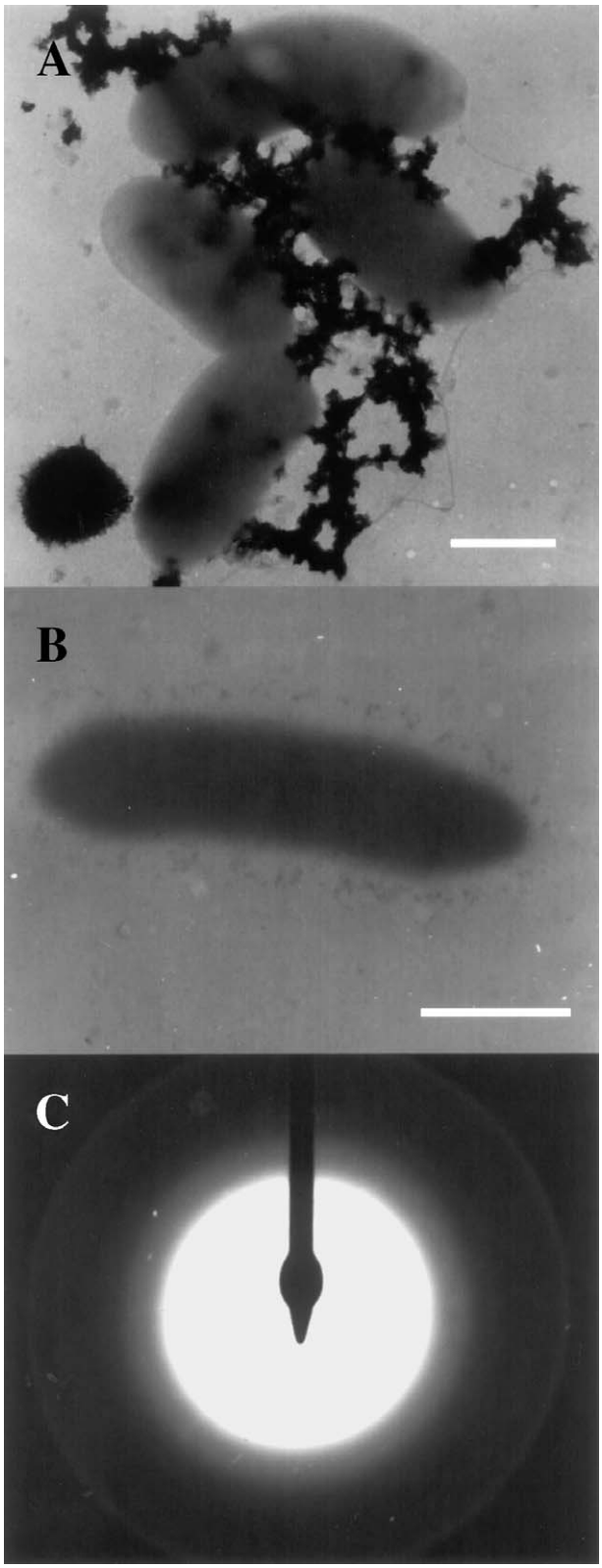

Fig. 7. TEM micrographs of whole mounts of bacteria cells before the addition of $Au(S_2O_3)_2^{3-}$ from Type 1 bacterial experiments: (A) in the systems containing iron; and (B) in the systems without iron. In the systems containing iron, the bacteria cells are associated with iron sulfide, while in the systems without iron, the bacteria surfaces are relatively clean. (C) TEM-SAED diffraction pattern of iron sulfide showing its amorphous nature. Scale bars in A and B are 0.5 μm.

experiments, Eh, pH, total concentrations of gold and iron were relatively constant during the experiments, and sulfide was not detected (Table 3).

3.2. TEM, SEM, and XRD

TEM observations on bacterial cells of Type 1 bacterial experiments before the addition of $Au(S_2O_3)_2^{3-}$ are presented in Fig. 7. The SRB cells were associated with fine-grained iron sulfide in the systems containing iron (Fig. 7A). Conversely, the bacterial cell surfaces in the SRB culture were "clean" in the systems without iron (Fig. 7B). XRD and TEM-SAED of the precipitated iron sulfide by bacteria demonstrated that it was non-crystalline (Figs. 7C and 8).

TEM and SEM observations on products of Type 1 bacterial experiments after the addition of $Au(S_2O_3)_2^{3-}$ are presented in Figs. 9–12. In the systems containing iron, nanoparticles of gold (<10 nm) were precipitated on bacterial cells, and sub-octahedral gold particles were precipitated near iron sulfide (Fig. 9A; indicated by arrows). The occurrence of gold was associated with iron sulfide (Fig. 9B–D). Gold nanoparticles (<25 nm) were dispersed in iron sulfide (Fig. 9C and D). In the systems without iron, gold was precipitated as nanoparticles on the bacterial cells and in the bulk solution (Fig. 10). In both systems containing iron and without iron, gold nanoparticles (<10 nm) were precipitated throughout the cytoplasm of the SRB sub-population of the culture (Fig. 11A and B), while some bacteria cells did not contain gold nanoparticles (Fig. 11C and D). Ultra-thin section TEM also revealed that nanoparticles of gold were deposited around some cells (Fig. 11E). TEM-EDS of ultra-thin section of the bacterial cells showed the occurrence of gold with sulfur (Fig. 11F). Ultimately, the precipitation of gold by SRB resulted in the formation of spherical aggregates containing octahedral gold, and individual octahedral and sub-octahedral gold crystal (Fig. 12). This octahedral gold yielded a TEM-SAED pattern consistent with crystalline gold (Fig. 12E).

4. Discussion

4.1. Bacterial and abiotic experiments

4.1.1. Type 1 bacterial versus the corresponding abiotic experiments

In the systems without iron, gold was precipitated in the presence of SRB (42–62%), while in the corresponding abiotic experiments, soluble gold was slightly reduced (3–4%) under similar conditions and duration (Fig. 3 and Table 2). These results demonstrated the role of SRB in gold precipitation from $Au(S_2O_3)_2^{3-}$ solutions. The role of SRB in the precipitation of gold from $Au(S_2O_3)_2^{3-}$ appears to be a much more involved process than simply lowering the redox conditions. The gold that was precipitated by SRB in these systems was found within the bacteria cells of a sub-population of the SRB enrichment, as shown in

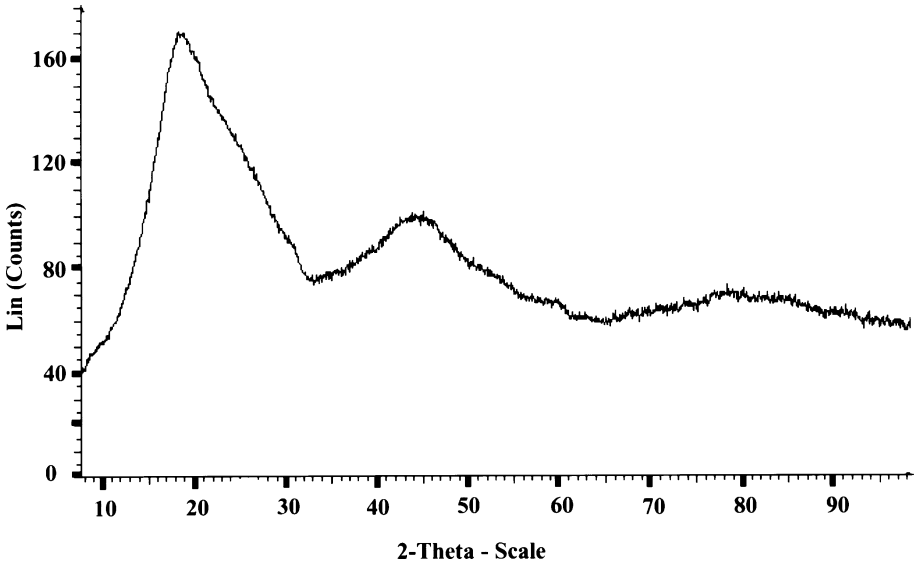

Fig. 8. XRD pattern of iron sulfide showing a broad peak of its amorphous nature.

Fig. 11. This suggests that the precipitation of gold from $Au(S_2O_3)_2^{3-}$ was a part of metabolic process of SRB utilizing thiosulfate (or $Au(S_2O_3)_2^{3-}$). The precipitation of gold nanoparticles (<10 nm) around the bacterial cells and not inside the cells suggests that SRB cells generated localized reducing conditions causing instability of $Au(S_2O_3)_2^{3-}$. The gold nanoparticles were ultimately formed larger gold particles (~0.5 to 7 μm) with sub-octahedral and octahedral shapes (Fig. 12).

Sulfide concentrations remained constant during the experiments, although it would be expected that these concentrations should have increased with time as a result of thiosulfate reduction to sulfide by SRB. The initial sulfide concentrations (time 0) in the systems without gold were lower than those in the systems with gold (Table 2). This suggests that the reduction of thiosulfate from $Au(S_2O_3)_2^{3-}$ occurred immediately after the addition of the gold solution. Under nutrient limiting conditions, i.e., no electron acceptor, stationary phase SRB would saturate the electron carriers available in each cell, which would allow for the rapid reduction of thiosulfate to sulfide after the addition of $Au(S_2O_3)_2^{3-}$.

In the systems containing iron, gold was precipitated in both bacterial and abiotic experiments; more gold was precipitated in the bacterial experiments than that in the corresponding abiotic experiments (Fig. 3 and Table 2). The presence of iron sulfide caused significant gold precipitation. The amount of initial iron sulfide was approximately four times higher in the bacterial experiments than that in the abiotic experiments, although at the completion of experiments, the amount of iron sulfide in both bacterial and abiotic experiments was similar with the exception for the 2.55 mM gold. The higher amount of initial iron sulfide in the bacterial experiments increased the amounts of gold precipitation. This demonstrates that the SRB promoted the precipitation of iron sulfide that further enhanced gold precipitation. The precipitation of gold nanoparticles inside the sub-population of SRB indicated that the role of SRB in gold precipitation not limited to the formation iron sulfide, which enhanced gold precipitation, but was also directly catalyzed this gold complex through metabolism. The growth of gold nanoparticles would ultimately have formed larger gold particles (Fig. 12).

4.1.2. Type 2 bacterial versus the corresponding abiotic experiments

In both systems containing iron and without iron, gold was only precipitated from solutions in the bacterial experiments, while in the corresponding abiotic experiments, gold was stable in solution (Figs. 5 and 6, Table 3). The addition of the SRB inoculum caused instability of $Au(S_2O_3)_2^{3-}$, while in the abiotic experiments, the absence of SRB did not promote the conditions that caused instability of soluble gold. In the bacterial experiments, gold was removed within 1 h after the addition of SRB inoculum, however, the reducing conditions in the bulk fluid phase was not generated until day 4 (Figs. 5 and 6). The SRB presumably generated localized reducing conditions causing instability of gold around the bacteria that further precipitated gold. While, the gold concentrations used in the Type 2 experiments appeared to be relatively low (<0.14 mM gold), the corresponding SRB population in the inoculum was five orders of magnitude lower than the Type 1 experiments (5×10^3 MPN/mL); yet the SRB were still able to reduce most of the soluble gold.

4.1.3. Type 1 versus Type 3 bacterial experiments

In the presence of live SRB culture (Type 1 bacterial experiments), soluble gold was precipitated from solutions, while in the presence of dead, autoclaved SRB, soluble gold was maintained in solutions. After SRB culture was autoclaved, Eh values increased from −0.17 to 0.4 V (Table 4), and the presence of initial sulfide or iron sulfide was

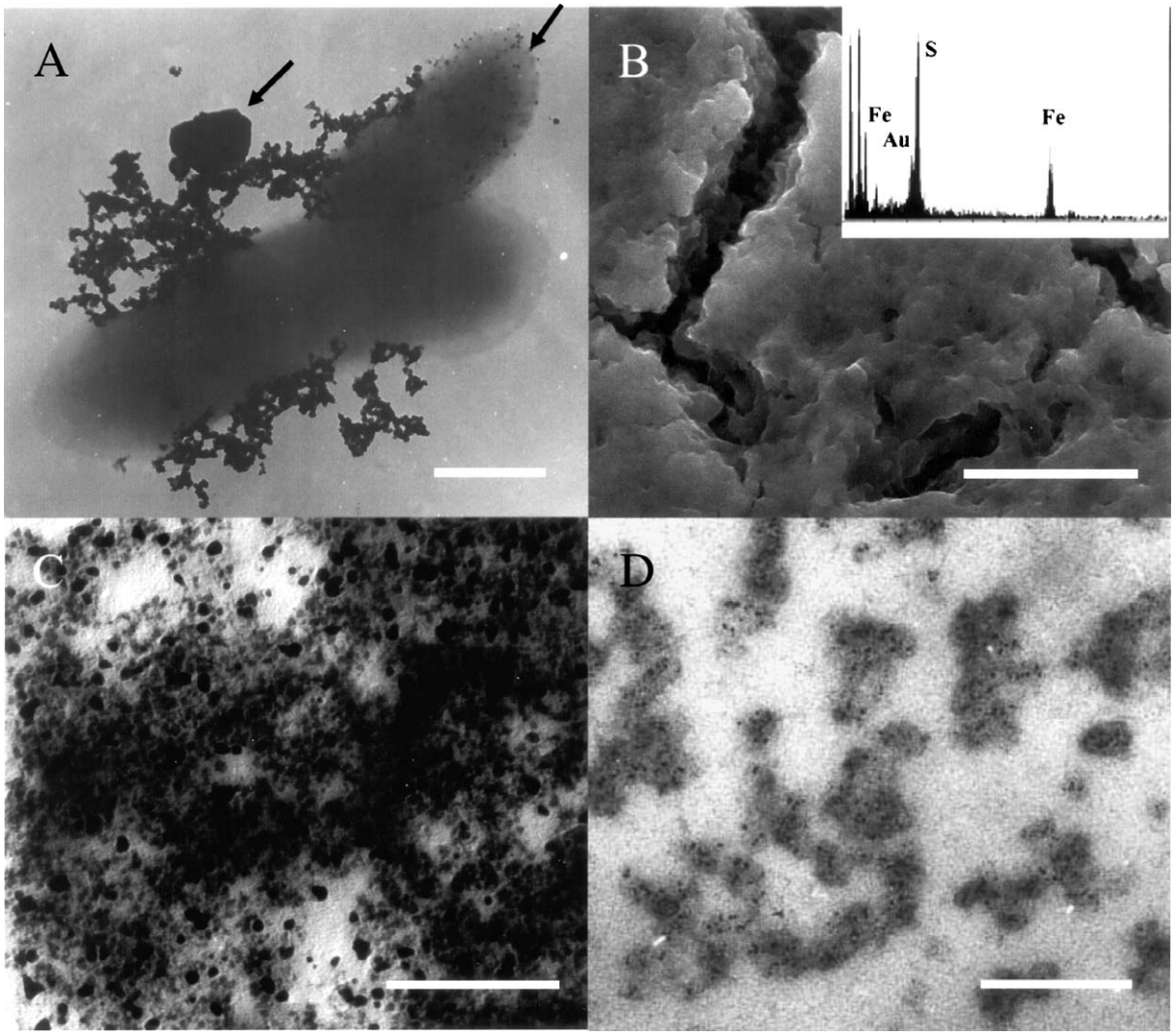

Fig. 9. TEM and SEM micrographs from Type 1 bacterial experiments containing iron at 2.55 mM gold and day 53. (A) A TEM micrograph of whole mounts of bacteria cells associated with iron sulfide and gold; the arrows indicate the location of gold; (B) A SEM micrograph of iron sulfide associated with gold (inset, SEM-EDS); (C) A TEM micrograph of whole mounts of iron sulfide containing dispersed gold nanoparticles; (D) An ultra-thin section TEM micrograph of the iron sulfide containing gold nanoparticles (from 'C'). Scale bars in A through D are 0.5, 5, 0.1, and 0.2 μm, respectively.

not longer detected, suggesting that the redox conditions were changed. Under relatively high Eh conditions and without the presence of SRB, $Au(S_2O_3)_2^{3-}$ was stable in solutions, and gold was not precipitated.

4.1.4. Type 1 versus Type 2 bacterial experiments

In Type 2 bacterial experiments, gold was precipitated from solution within 1 h after the addition of the SRB inoculum. In Type 2 bacterial experiments, final gold concentrations were lower ($\leqslant$0.14 mM gold) than those in Type 1 bacterial experiments ($\leqslant$2.55 mM gold); the SRB population in Type 2 bacterial experiments was five orders of magnitude lower than that in Type 1 experiments (Tables 2 and 3). Intuitively, the more rapid removal of gold from solution in the Type 1 bacterial experiments versus Type 2 experiments would be expected. However, the experimental results showed the reverse conditions in which the precipitation of gold was more rapid in Type 2 bacterial experiments than in Type 1 experiments. This suggests that in Type 1 bacterial experiments, another form of soluble gold, i.e., an organo-gold or gold sulfide complex could be present. At higher gold concentrations ($\geqslant$0.22 mM), $Au(S_2O_3)_2^{3-}$ exhibited toxicity towards the SRB inoculum in the Type 2 experiments, and as a result the SRB did not grow. The level of gold toxicity for SRB is $\sim$0.22 mM gold in the form of $Au(S_2O_3)_2^{3-}$, and similar level of gold toxicity was also observed for thiosulfate-oxidizing bacteria (Lengke and Southam, 2005). Although the SRB inoculum could not grow in the presence of high gold concentrations, the stationary phase of SRB culture could stand the influx of high concentrations of gold ($\leqslant$2.55 mM gold).

The gold concentrations used in this laboratory model were much higher than those encountered in natural systems. In natural systems, where gold concentrations are

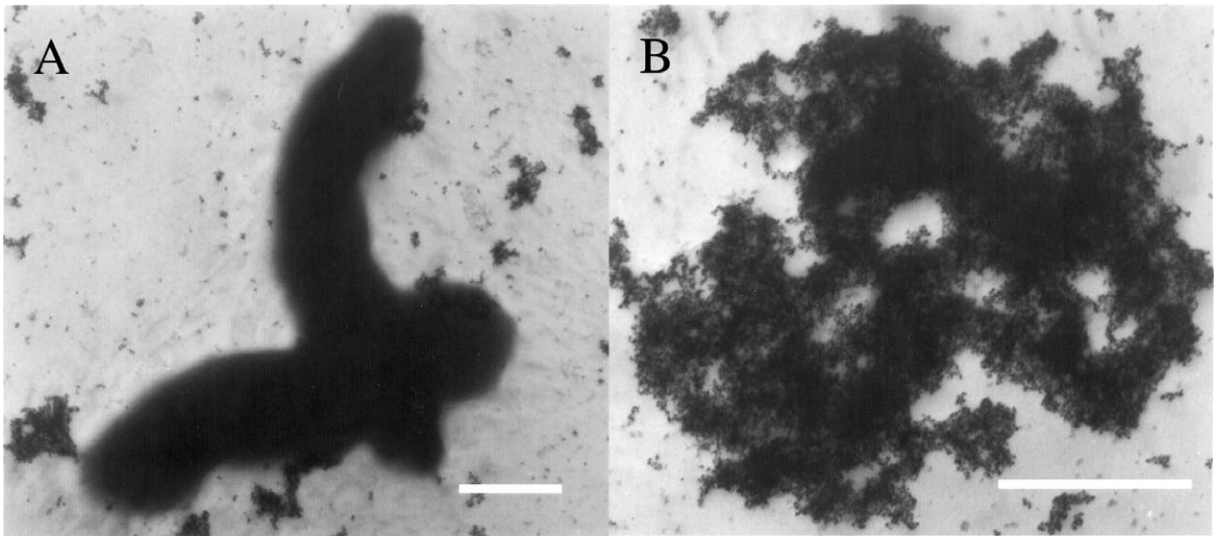

Fig. 10. TEM micrographs of whole mounts of (A) bacteria cells associated with nanoparticles of gold on the cell surfaces, and (B) nanoparticles of gold in the bulk solution in Type 1 bacterial experiments without iron at 2.55 mM gold and day 53. Scale bars in A and B are 0.5 μm.

at the ppb level (Bowell et al., 1993; Aylmore and Muir, 2001), gold toxicity is not likely to affect the biogeochemical process.

4.2. Possible mechanisms of gold precipitation by SRB

Based on the discussion above, the precipitation of gold from $Au(S_2O_3)_2^{3-}$ by SRB was caused by three possible mechanisms: (1) iron sulfide, (2) localized reducing conditions, and (3) a metabolic process.

The role of sulfide mineral surfaces, e.g., arsenic sulfide, stibnite sulfide, pyrite, iron sulfide, and arsenopyrite, in scavenging gold has been reported (e.g., Jean and Bancroft, 1985; Renders and Seward, 1989; Schoonen et al., 1992; Mycroft et al., 1995; Maddox et al., 1998; Widler and Seward, 1996, 1998, 2002). Studies of gold(III)-chloride adsorption on pyrite involved a two-step reaction by forming a metastable Au(I) surface complex, and further reduced to Au(0) (Jean and Bancroft, 1985; Mycroft et al., 1995; Maddox et al., 1998). The adsorption of gold(I)-hydrosulfide complexes ($AuHS^0$) has been determined on iron sulfide and pyrite surfaces leading to the reduction of Au(I) to Au(0) (Widler and Seward, 1996, 1998, 2002). Iron sulfide generated by SRB could have adsorbed $Au(S_2O_3)_2^{3-}$ onto freshly forming surfaces leading to the precipitation of gold. The presence of dispersed gold within iron sulfide demonstrated that iron sulfide was able to reduce Au(I) to Au(0).

The localized reducing conditions generated by SRB that caused gold precipitation were associated with metabolism. A simplified model of thiosulfate utilization by SRB is presented in Fig. 13. Thiosulfate and $Au(S_2O_3)_2^{3-}$ enter the bacteria cells through pores at cell surfaces. Thiosulfate is reduced by a metabolic process and can be represented by the half reaction as follows:

$$S_2O_3^{2-} + 8H^+ + 8e^- \rightarrow 2HS^- + 3H_2O \quad (3)$$

The reduction of thiosulfate to hydrogen sulfide (HS^-) by SRB is not a direct pathway, but the process involves intermediate steps by forming other sulfur species. For simplicity, hydrogen sulfide is written as sulfide (S^{2-}) in reactions 4 and 5. Thiosulfate reduction proceeded through formation of sulfide (S^{2-}) and sulfite SO_3^{2-} (Akagi, 1995; reaction 4). Sulfite is reduced to sulfide by two possible mechanisms (Akagi, 1995). One hypothesis is that the reduction occurs without the formation of any intermediate species, and another hypothesis proposes the formation of trithionate and thiosulfate (reaction 5).

$$S_2O_3^{2-} + 2e^- \leftrightarrow S^{2-} + SO_3^{2-} \quad (4)$$

$$3SO_3^{2-} + 2e^- \leftrightarrow S_3O_6^{2-} + 2e^- \leftrightarrow S_2O_3^{2-} + SO_3^{2-} + 2e^- \leftrightarrow S^{2-} + SO_3^{2-} \quad (5)$$

Hydrogen sulfide (HS^-), as the end product of metabolism, is released through outer membrane pores. The released of hydrogen sulfide decreases redox conditions around the bacteria cells, forming localized reducing conditions.

When $Au(S_2O_3)_2^{3-}$ was degraded by SRB, elemental gold was precipitated as nanoparticles (<10 nm) inside a sub-population of SRB cells (Fig. 11A and B). The presence of gold nanoparticles throughout cytoplasm suggests that $Au(S_2O_3)_2^{3-}$ entered the bacterial cells as a complex, and degraded as follows:

$$Au(S_2O_3)_2^{3-} \rightarrow Au^+ + 2S_2O_3^{2-} \quad (6)$$

While thiosulfate is metabolically degraded to hydrogen sulfide, Au^+ could be reduced by an intracellular electron donor or exported by a membrane transporter system (Nies and Silver, 1995). However, based on the presence of gold nanoparticles inside the cytoplasm, Au^+ is presumably reduced to Au^0.

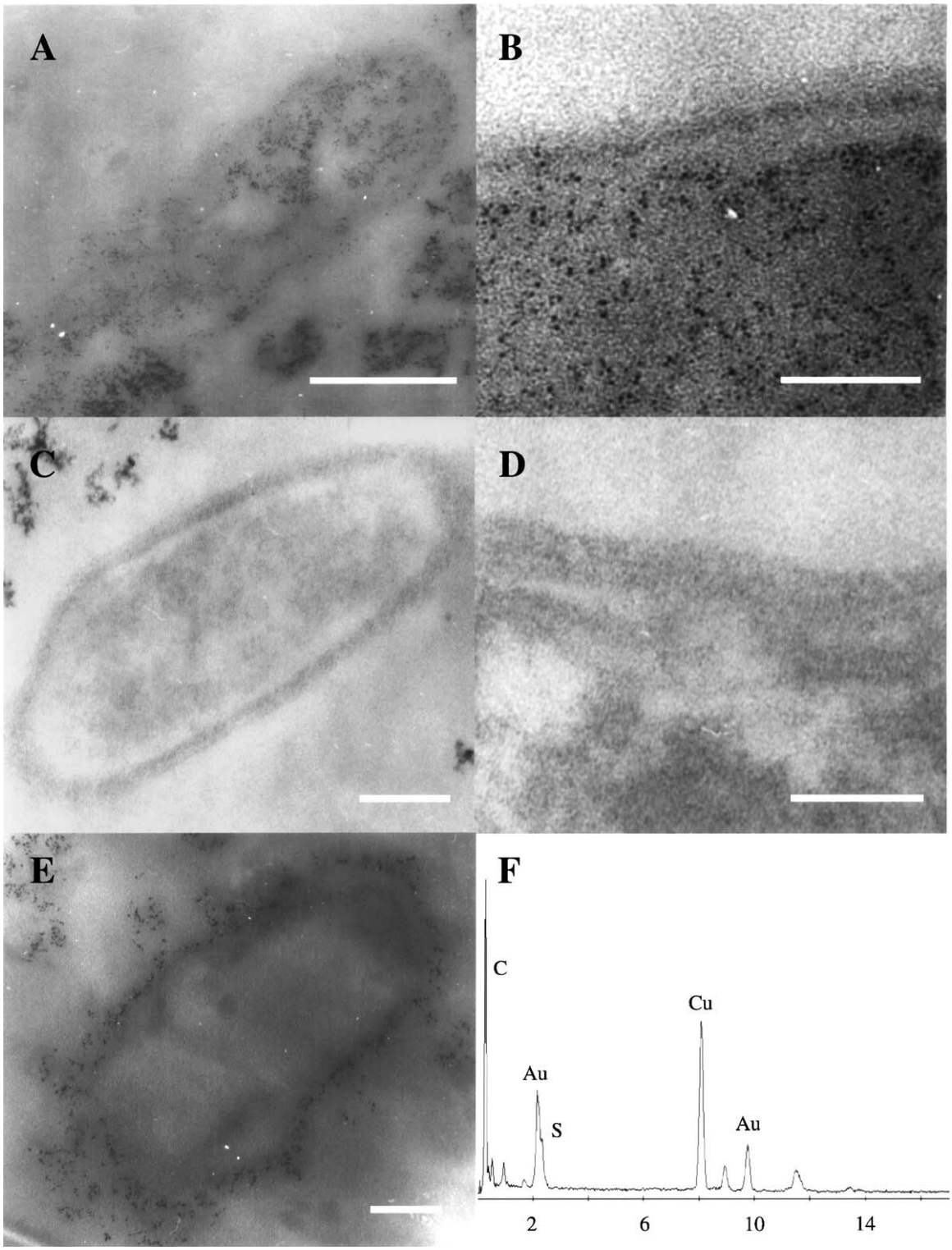

Fig. 11. TEM micrographs of ultra-thin section of bacteria cells from Type 1 bacterial experiments at 2.55 mM gold and day 53: (A) gold nanoparticles precipitated throughout the cell and (B) within the bacterial cytoplasm in the systems containing iron; (C and D) the absence of gold nanoparticles within the cell; and (E) gold nanoparticles precipitated around the cell in the systems without iron; (F) TEM-EDS from panel E. Scale bars in A through E are 0.5, 0.1, 0.1, 0.05, and 0.2 μm, respectively.

The formation of gold nanoparticles around the bacterial cells (Fig. 11E) possibly caused by the localized reducing conditions that precipitated gold from $Au(S_2O_3)_2^{3-}$ solutions. In late stationary phase or death phase, the uptake of Au^+ into the cytoplasm and its reduction to Au^0 damaged the 'biochemical machinery' of the cell, possibly limiting the export of Au^+ from the cytoplasm. The gold nanoparticles,

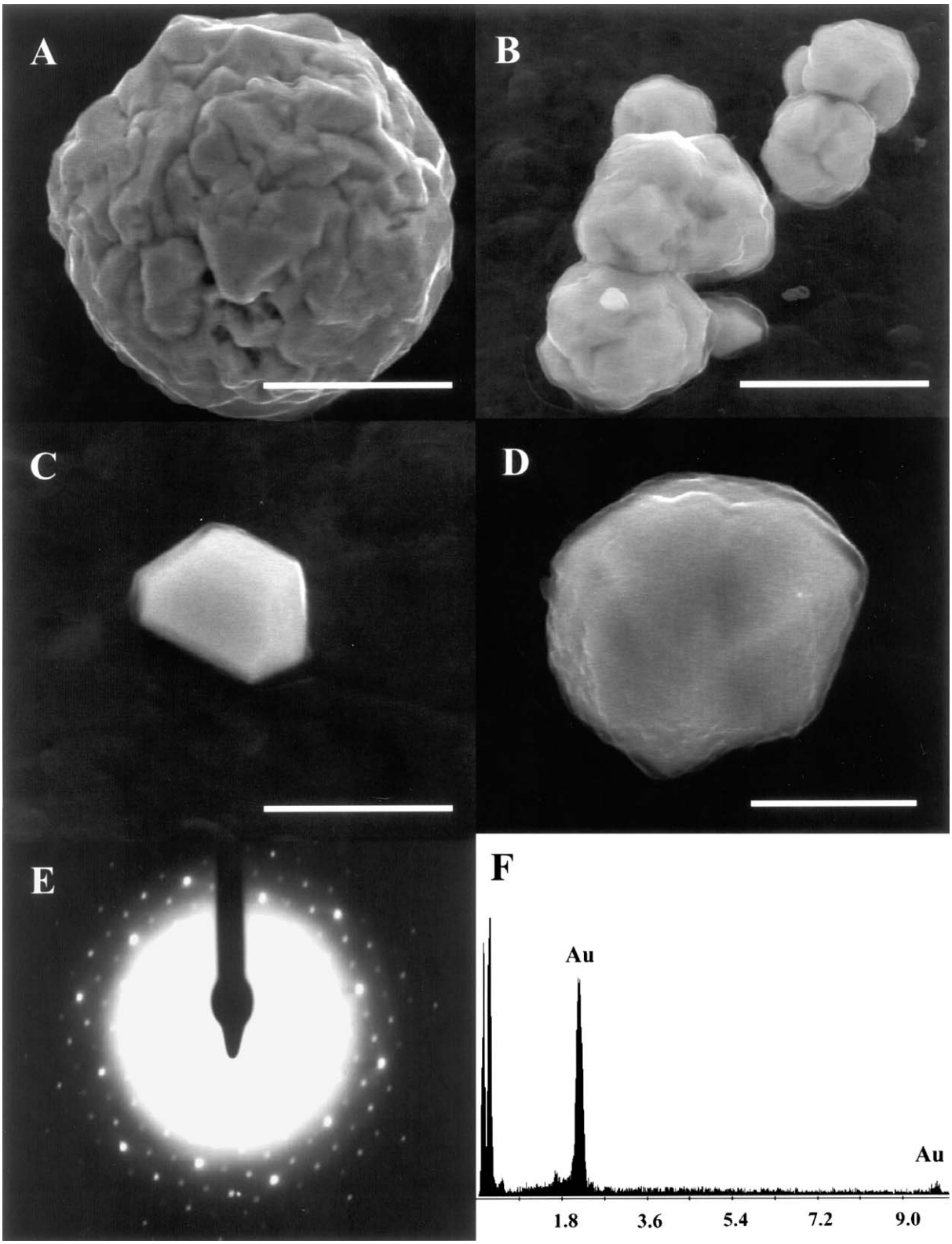

Fig. 12. SEM micrographs of gold particles from Type 1 bacterial experiments at 2.55 mM gold and day 53 in the systems without iron: (A and B) roughly spherical gold possessing octahedral and sub-octahedral habits; (C) single octahedral gold crystals; and (D) single sub-octahedral gold crystal; (E) TEM-SAED twinned diffraction pattern consistent with crystalline gold; (F) SEM-EDS from panel A. Scale bars in A through D are 3, 1.5, 0.75, and 0.5 μm, respectively.

initially precipitated inside the cytoplasm, could be released to the bulk solution due to the lysis of some of the SRB. The gold nanoparticles were then formed sub-octahedral and octahedral gold, and spherical aggregates of octahedral gold having a micrometer-size range (Fig. 12).

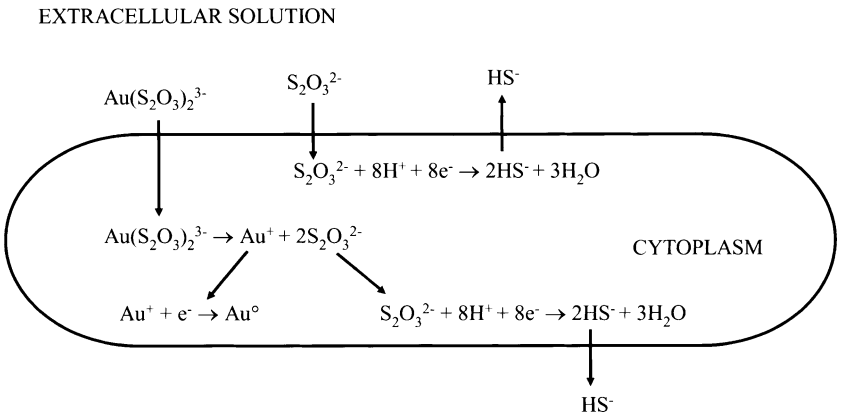

Fig. 13. A model of thiosulfate and gold(I)-thiosulfate complex ($Au(S_2O_3)_2^{3-}$) utilization by SRB as a source of energy. Thiosulfate and $Au(S_2O_3)_2^{3-}$ are entered the bacterial cell through outer membrane pores, and the metabolism product, i.e., hydrogen sulfide (HS^-) is released through the pores. When thiosulfate from $Au(S_2O_3)_2^{3-}$ is consumed for metabolism, and Au^+ is released, and presumably reduced to Au^0 inside the cytoplasm.

This present study demonstrated that the dissociation of $Au(S_2O_3)_2^{3-}$ is an active process that is catalyzed by the dissimilatory SRB or the result of bacterial reduction of free thiosulfate which resulted in chemical dissociation of $Au(S_2O_3)_2^{3-}$. Previous studies by Southam and Beveridge (1994, 1996) have demonstrated that octahedral gold was formed by the interaction between $AuCl_4^-$ and organic acids derived from dead bacteria, and the presence of active (live) bacteria was not necessary to promote gold precipitation. However, this study shows that only active (live) SRB promoted gold diagenesis. Similar results were also observed for thiosulfate-oxidizing bacteria in which their presence was essential in the precipitation of gold (Lengke and Southam, 2005).

4.3. Implications for the formation of supergene gold

This study shows that the interaction of SRB and $Au(S_2O_3)_2^{3-}$ solution results in gold precipitation, and more specifically, in distinctive and characteristic morphology for gold particles. Sulfate-reducing bacteria (SRB) were able to utilize $Au(S_2O_3)_2^{3-}$ as an energy source, to promote localized reducing conditions, and to generate iron sulfide (if iron is present), as a result, gold was precipitated both intracellularly (<10 nm) and extracellularly (<25 nm to 8 μm), the latter with distinct sub-octahedral and octahedral shapes, and also nanoparticles.

The occurrence of nanoparticles of gold has been observed in natural environments, and is generally called as "invisible gold." The formation of "invisible gold" has been attributed by adsorption processes of soluble gold on sulfide surfaces (e.g., Renders and Seward, 1989; Widler and Seward, 1998, 2002). The association of gold and amorphous iron sulfide has been reported in active Ladolam epithermal deposit on Lihir Island, Papua New Guinea (Widler and Seward, 2002). Although chemi-sorption is important in the deposition of gold, this study shows that SRB could contribute to the direct precipitation of secondary gold through reduction processes.

Octahedral gold grains have been identified as an important characteristic of secondary gold and observed in Mother Lode, California (Leicht, 1982), weathered Archean metamorphic rocks in Western Australia (Wilson, 1984), and in the Witwatersrand Au deposit, South Africa (Frimmel et al., 1993; Minter et al., 1993). Although high temperature and high pressure conditions can result in the formation of octahedral gold (Frimmel et al., 1993), the ability of SRB to form octahedral gold from $Au(S_2O_3)_2^{3-}$ is significant. Thiosulfate is an important gold complexing agent in natural systems (Saunders, 1989; Vlassopoulos and Wood, 1990), and this complex was responsible for the formation of secondary gold in low temperature placer gold and supergene gold systems, e.g., the San Luis Range, Argentina; the Waimumu, New Zealand; the Wau Wau gold field, Papua New Guinea and the Ashanti Mine, Ghana (Clough and Craw, 1989; Saunders, 1989; Bowell et al., 1993; Aylmore and Muir, 2001; Márquez-Zavalía et al., 2004). The ubiquitous nature of bacteria in near-surface environments (Lovley and Chapelle, 1995) and the rapid precipitation of gold by the SRB enrichment, suggest that these bacteria could be responsible for the enrichment of gold in natural systems. In this model, bioaccumulation of gold and formation of secondary gold minerals from $Au(S_2O_3)_2^{3-}$ are responsible for the formation of octahedral gold, typically associated with placer or supergene gold nuggets in natural systems.

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