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WINOGRADSKY REVIEW

The geomicrobiology of gold

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Microorganisms capable of actively solubilizing and precipitating gold appear to play a larger role in the biogeochemical cycling of gold than previously believed. Recent research suggests that bacteria and archaea are involved in every step of the biogeochemical cycle of gold, from the formation of primary mineralization in hydrothermal and deep subsurface systems to its solubilization, dispersion and re-concentration as secondary gold under surface conditions. Enzymatically catalysed precipitation of gold has been observed in thermophilic and hyperthermophilic bacteria and archaea (for example, *Thermotoga maritime, Pyrobaculum islandicum*), and their activity led to the formation of gold- and silver-bearing sinters in New Zealand's hot spring systems. Sulphate-reducing bacteria (SRB), for example, *Desulfovibrio* sp., may be involved in the formation of gold-bearing sulphide minerals in deep subsurface environments; over geological timescales this may contribute to the formation of economic deposits. Iron- and sulphur-oxidizing bacteria (for example, *Acidothiobacillus ferrooxidans, A. thiooxidans*) are known to breakdown gold-hosting sulphide minerals in zones of primary mineralization, and release associated gold in the process. These and other bacteria (for example, actinobacteria) produce thiosulphate, which is known to oxidize gold and form stable, transportable complexes. Other microbial processes, for example, excretion of amino acids and cyanide, may control gold solubilization in auriferous top- and rhizosphere soils. A number of bacteria and archaea are capable of actively catalysing the precipitation of toxic gold(I/III) complexes. Reductive precipitation of these complexes may improve survival rates of bacterial populations that are capable of (1) detoxifying the immediate cell environment by detecting, excreting and reducing gold complexes, possibly using P-type ATPase efflux pumps as well as membrane vesicles (for example, *Salmonella enterica*, *Cupriavidus* (*Ralstonia*) *metallidurans*, *Plectonema boryanum*); (2) gaining metabolic energy by utilizing gold-complexing ligands (for example, thiosulphate by *A. ferrooxidans*) or (3) using gold as metal centre in enzymes (*Micrococcus luteus*). *C. metallidurans* containing biofilms were detected on gold grains from two Australian sites, indicating that gold bioaccumulation may lead to gold biomineralization by forming secondary 'bacterioform' gold. Formation of secondary octahedral gold crystals from gold(III) chloride solution, was promoted by a cyanobacterium (*P. boryanum*) via an amorphous gold(I) sulphide intermediate. 'Bacterioform' gold and secondary gold crystals are common in quartz pebble conglomerates (QPC), where they are often associated with bituminous organic matter possibly derived from cyanobacteria. This may suggest that cyanobacteria have played a role in the formation of the Witwatersrand QPC, the world's largest gold deposit.
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Introduction

Microorganisms play an important role in the geochemical cycling of metals. These metal cycles are driven by microorganisms, because metals are essential for microbial nutrition (for example, Mg, Na, Fe, Co, Cu, Mo, Ni, W, V, Zn; Madigan and Martinko, 2006). A number of metal ions are oxidized or reduced in catabolic reactions to gain energy (for example, As(III/V), Fe(II/III), Mn(II/IV), V(IV/V), Se(IV/VI), U(IV/VI); Tebo and Obraztsova, 1998; Stolz and Oremland, 1999; Ehrlich, 2002; Lloyd, 2003; Tebo *et al.*, 2005; Madigan and Martinko, 2006). Metal ions (for example, Ag^{+}, Hg^{2+}, Cd^{2+}, Co^{2+}, CrO_4^{2-}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}) can also cause toxicity through the displacement of essential metals from their native binding sites, because they possess a greater affinity to thiol-containing groups and oxygen sites than essential metals (Poole and Gadd, 1989; Nies, 1999; Bruins

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et al., 2000; Lloyd, 2003; Silver and Phung, 2005). Solubilization, precipitation, fractionation and speciation of metals and metal ions are also indirectly influenced by microbial activity, for example, through their influence on environmental redox conditions and pH, formation of complexing ligands and bioaccumulation (Stone, 1997; Ehrlich, 2002; Lloyd, 2003).

These mechanisms, which are widely accepted as drivers of environmental metal cycling, are now being examined using the latest molecular microbiology techniques (for example, whole-genome sequencing and RNA-expression microarrays as well as metagenomic and proteomic approaches) to link microbial processes, populations and communities directly to element transformations in the environment (Newman and Banfield, 2002; Handelsman, 2004; Tyson *et al.*, 2004; Ram *et al.*, 2005; Valenzuela *et al.*, 2006; Whitaker and Banfield, 2006). The use of metagenomic and proteomic techniques has elucidated the complex interactions in a microbial community that underpins the generation of acid mine drainage and metal mobilization at the Richmond Mine in California (Tyson *et al.*, 2004; Ram *et al.*, 2005). This research has also led to an understanding of the adaptation mechanisms these communities use to survive under extremely acidic and toxic metal-rich conditions (Dopson *et al.*, 2003; Druschel *et al.*, 2004; Tyson *et al.*, 2004; Ram *et al.*, 2005). In other studies biochemical pathways of microbial metal and metalloid transformations (for example, oxidation, reduction, methylation, complexation and biomineralization of Fe(II/III), Mn(II/IV), As(III/V), Se(IV/VI), V(IV/V) and U(IV/VI) have been linked to biogeochemical metal cycling in sediments, soils and other weathered materials from polar, temperate, arid and tropical regions (Barns and Nierzwicki-Bauer, 1997; Stolz and Oremland, 1999; Holden and Adams, 2003; Gadd, 2004; Islam *et al.*, 2004; DiChristina *et al.*, 2005; Nelson and Methé, 2005; Wilkins *et al.*, 2006; Akob *et al.*, 2007; Edwards *et al.*, 2007; Fitzgerald *et al.*, 2007). In another approach *Cupriavidus* (formerly known as *Ralstonia, Wautersia* and *Alcaligenes*) *metallidurans* was used as a model organism to study the metal resistance mechanisms in bacteria, and a complex network of genetic and proteomic responses that regulate the resistance to a number of toxic metal ions, for example, Ag^{+}, Hg^{2+}, Cd^{2+}, Co^{2+}, CrO_4^{2-}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, has been described (Mergeay *et al.*, 1985, 2003; Nies, 1992, 1995, 1999, 2003; Nies and Silver, 1995; Nies and Brown, 1998; Grosse *et al.*, 2004; Monchy *et al.*, 2006).

In contrast, to the metals mentioned above, gold is extremely rare, inert, non-essential, unstable as a free ion in aqueous solution under surface conditions, and as a complex, it can be highly toxic to organisms (Boyle, 1979; Witkiewicz and Shaw, 1981; Karthikeyan and Beveridge, 2002). Research into microbial processes affecting the cycling of gold as well as the physiological and biochemical responses of microorganisms to toxic gold complexes has been limited, and as a result its biogeochemical cycling and geomicrobiology are poorly understood. However, a microbially driven biogeochemical cycle of gold has been proposed, and in a number of laboratory and field studies individual aspects of this cycle have been assessed (for example, Korobushkina *et al.*, 1983; Savvaidis *et al.*, 1998; Mossman *et al.*, 1999). Laboratory studies have shown that some archaea, bacteria, fungi and yeasts are able to solubilize and precipitate gold under *in vitro* conditions, but these experiments give only limited insights into gold solubilization and precipitation catalysed by complex microbial communities in natural environments. In a field-based approach, the morphology of secondary gold grains that display 'bacterioform' structures has been used as evidence for microbial gold precipitation and biomineralization in the environment (Watterson, 1992). However, analogous gold morphologies were also produced abiotically, and are therefore not sufficient proof for the bacterial origin of 'bacterioform' gold (Watterson, 1994). To obtain conclusive evidence for microbially mediated environmental cycling of gold, a combination of laboratory-based experiments and field observations supported by the state-of-the-art microscopy techniques (for example, high-resolution scanning electron microscopy (SEM), transmission electron microscopy (TEM), confocal scanning laser microscopy (CSLM)), spectroscopy ((X-ray absorption spectroscopy (XAS), inductively coupled plasma mass spectroscopy (ICP-MS)) and molecular microbial techniques (for example, 16S rDNA PCR-DGGE, shotgun cloning and sequencing, expression of functional genes) have been conducted.

The aim of this review is to integrate these recent results with the results of the earlier studies in order to trace the path of gold and associated microbial processes and populations through the environment (Figure 1). After providing an overview of the geochemical properties and constraints of gold in hydrothermal and supergene systems, the influence of bacteria and archaea on primary deposit formation as well as solubilization, redistribution and precipitation in surface environments are reviewed. Then, the microbially mediated formation of secondary octahedral gold crystals and 'bacterioform' gold, which are common in quartz pebble conglomerates (QPC) deposits (for example, the largest known gold deposit, the Witwatersrand QPC) are discussed because recent results have rekindled the debate of whether or not these are products of microbial processes. Thus, this review will provide the background for future research that will aim at directly linking the activities of complex microbial communities with gold cycling using advanced genomic, proteomic and spectroscopic techniques.

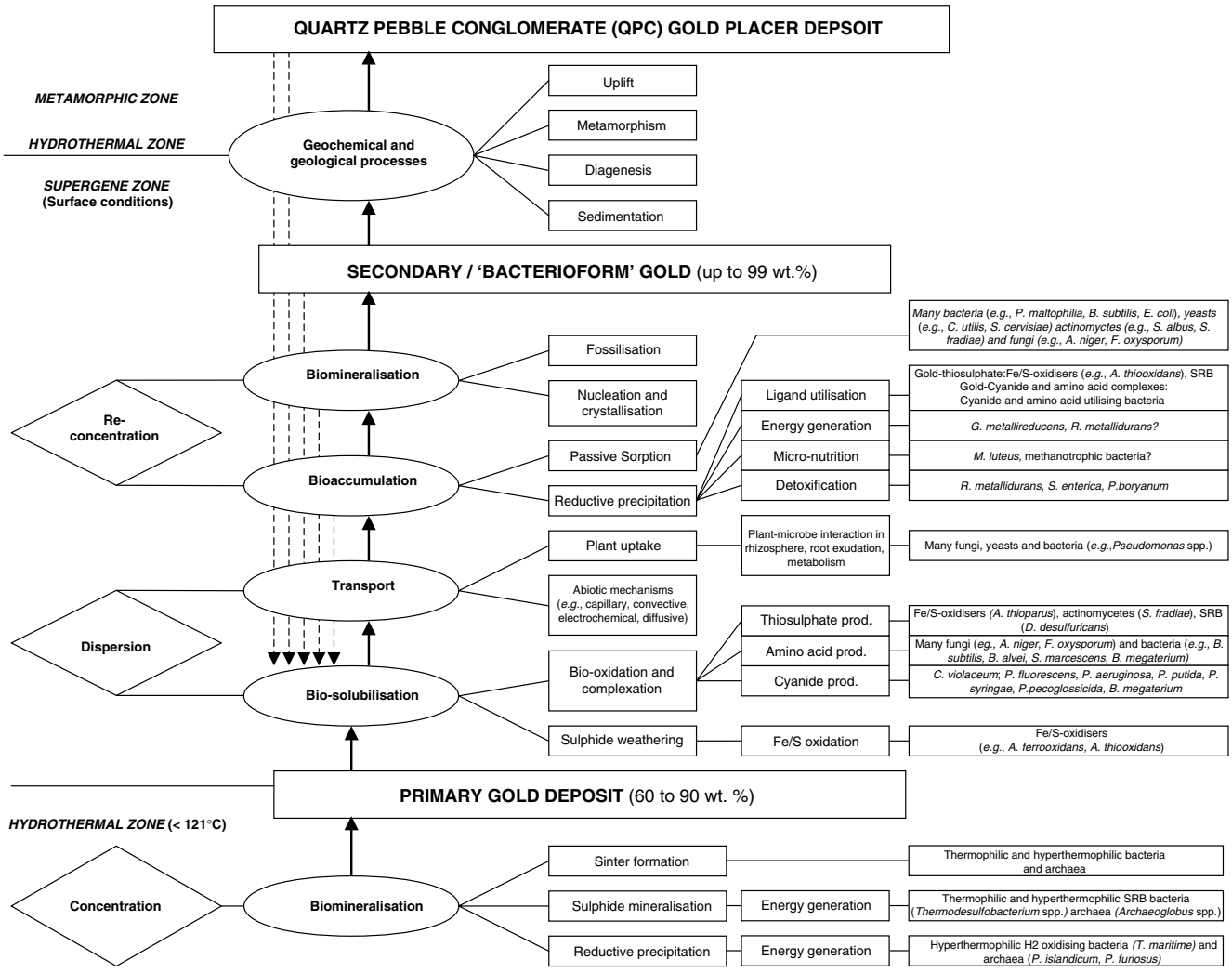

Figure 1 Schematic model for the biogeochemical cycling of gold linking gold dispersion and concentration in the environment to biological processes, microbial populations and selected abiotic mechanisms.

The geochemical characteristics of gold

Gold is one of the ten rarest elements in the Earth's crust with an average concentration of $5\,ng\,g^{-1}$ (solid material), and a concentration range from 0.0197 to $0.197\,\mu g\,l^{-1}$ in natural waters (Goldschmidt, 1954; McHugh, 1988). However, gold is not uniformly distributed and is often highly enriched in mineralized zones, where it may form economic primary deposits (for example, skarn type-, vein type- and disseminated deposits; Boyle, 1979). The deposition of gold in primary deposits usually occurs via metal-rich hydrothermal fluids, which circulate in open spaces within rocks and deposit gold as consequence of cooling or boiling (Morteani, 1999). Native primary gold is commonly present as alloys with Ag, Cu, Al, Fe, Bi, Pb, Zn, Pd or Pt, with gold concentration ranging from 50 to 80 wt% (Boyle, 1979).

Due to its low solubility in aqueous solution, its speciation and complexation is commonly deduced from thermodynamic calculations, geochemical models, laboratory studies and field observations (Boyle, 1979, Gray, 1998). In hydrothermal fluids gold is chemically mobile as complexes with sulphide and bisulphide (for example, $[Au(HS)^0]$, $[Au(HS_2)^-]$, $[Au_2S_2^{2-}]$; Renders and Seward, 1989; Benning and Seward, 1996; Gammons and Williams-Jones, 1997; Gilbert *et al.*, 1998). Deposition from these solutions leads to the formation of gold-containing sulphide minerals (for example, pyrite and arsenopyrite), in which gold is either finely dispersed in crystal lattices or occurs as solid inclusions (Boyle, 1979; King, 2002). The chemical mobility of gold in the supergene zone is linked to the weathering of these sulphide minerals, and its subsequent oxidation and complexation (Boyle, 1979; Southam and Saunders, 2005). Under surface conditions, gold occurs in aqueous 'solution' as metal colloid (0), and aurous (+I) and auric (+III) complexes, because standard redox potentials of Au^+ (1.68 V) and Au^{3+} (1.50 V) exceed that of water

(1.23 V), which makes the existence of free gold ions thermodynamically unfavourable (Boyle, 1979; Vlassopoulos *et al.*, 1990a). Based on thermodynamic calculations and natural abundances of possible ligands, aqueous gold(I/III) complexes with thiosulphate and chloride appear to be the most important complexes in waters that contain little dissolved organic matter (DOM; Boyle, 1979; Mann, 1984; Vlassopoulos *et al.*, 1990a; Benedetti and Boulegue, 1991; Gray, 1998). Thiosulphate has been shown to readily solubilize gold, and the resulting gold(I) thiosulphate complex [$Au(S_2O_3)_2^{3-}$] is stable from mildly acid to highly alkaline pH, and moderately oxidizing to reducing conditions (Mineyev, 1976; Goldhaber, 1983; Webster, 1986). Thiosulphate is produced during the (bio)oxidation of sulphide minerals, and thus is likely to dominate in groundwater surrounding gold-bearing sulphide deposits (Stoffregen, 1986). Oxidizing groundwater with high dissolved chloride contents, common in arid and semi-arid zones, may also solubilize gold, leading to the formation of gold(I/III) chloride complexes ([$AuCl_2^-$], [$AuCl_4^-$]; Krauskopf, 1951; Gray, 1998). However, unlike the gold(III) chloride complex, the gold(I)-chloride complex ($AuCl_2^-$) is not be stable at low temperature (<100 °C) under oxidizing condition (Farges *et al.*, 1991; Pan and Wood, 1991; Gammons *et al.*, 1997).

Gold also forms complexes with organic ligands; a characteristic of Group IB metals is their ability to strongly bind to organic matter (Boyle, 1979; Vlassopoulos *et al.*, 1990b; Gray *et al.*, 1998). Thus, organic gold complexes may be important in soil solutions with high concentrations of DOM. However, due to the complexity of DOM, researchers have contradicting results in solubilization/precipitation experiments. Organic acids (that is, humic and fulvic acids, amino acids and carboxylic acids) have been shown to promote the solubilization of native gold in some experiments (Freise, 1931; Baker, 1973, 1978; Boyle *et al.*, 1975; Korobushkina *et al.*, 1983; Varshal *et al.*, 1984). However, in other experiments native gold was not oxidized and the formation of gold colloids and sols from gold(I/III) complexes was promoted (Fetzer, 1934, 1946; Ong and Swanson, 1969; Fisher *et al.*, 1974; Gray *et al.*, 1998). The interaction of gold and organic matter involves mostly electron donor elements, such as N, O or S, rather than C (Housecroft, 1993, 1997a, b). Vlassopoulos *et al.* (1990b) showed that gold binds preferentially to organic S under reducing conditions, whereas under oxidizing conditions it binds mostly to organic N and C. Organo-gold complexes can be present in aqueous solution as gold(I) cyanide complexes (Housecroft, 1993, 1997a, b). Gold(I) forms a strong complex with cyanide [$Au(CN)_2^-$] that is stable over a wide range of Eh-pH conditions (Boyle, 1979; Gray, 1998).

Sorption of gold complexes and colloids to organic matter, clays, Fe and Mn minerals as well as bioaccumulation and biomineralization may lead to the formation of secondary gold particles, which are often observed close to primary deposits (see below; Boyle, 1979; Goldhaber, 1983, Webster and Mann, 1984; Webster, 1986; Lawrance and Griffin, 1994; Gray, 1998). Secondary gold is generally much finer (up to 99 wt% of gold) compared to primary gold, and the individual gold aggregates are often larger than in potential source rocks (Wilson, 1984; Watterson, 1992; Mossman *et al.*, 1999).

Microbial influence on the formation of primary mineralization

Bacteria and archaea are found in surface and subsurface environments up to a depth of several kilometres, and are only limited by water availability and ambient temperature (<121 °C; Inagaki *et al.*, 2002; Baker *et al.*, 2003; Kashefi and Lovley, 2003; Navarro-Gonzalez *et al.*, 2003; Fredrickson and Balkwill, 2006; Teske, 2006). In ultra-deep gold mines (at depths of up to 5 km below land surface) sulphate-reducing bacteria (SRB; for example, *Desulfotomaculum* sp.) are common (Moser *et al.*, 2005), and may contribute to the formation of gold-containing sulphide minerals (Lengke and Southam, 2006, 2007). SRB are anaerobic, heterotrophic bacteria that use the dissimilatory sulphite reductase (*Dsr*) pathway for the reduction of sulphur compounds (for example, sulphate and thiosulphate) to hydrogen sulphide (H_2S), which is toxic, because it inhibits the function of iron-containing cytochromes (Truong *et al.*, 2006). Free sulphide (that is, present as H_2S, HS^- or S^{2-} depending on pH) is detoxified through precipitation with metal ions, such as Fe^{2+}, leading to the formation of metal sulphides, such as pyrite (Donald and Southam, 1999). *Desulfovibrio* sp. have been shown to reduce the thiosulphate from gold thiosulphate complexes; this destabilizes the gold in solution, which may then be incorporated into the newly forming sulphide minerals (Lengke and Southam, 2006). Thus, this process may contribute to the formation of economic gold deposits, if it continues over geological timescales, provided nutrients and gold(I) thiosulphate are provided to the system.

The presence of bacteria and archaea in hydrothermal systems, such as hydrothermal vents and hot springs, has been documented highlighting the growth of these organisms under extreme conditions (Barns *et al.*, 1994; Barns and Nierzwicki-Bauer, 1997; Sievert *et al.*, 2000; Kashefi and Lovley, 2003; Csotonyi *et al.*, 2006). Hydrothermal vents are geological formations that release altered seawater, which has been heated to temperatures of up to 400 °C by subterranean magma pockets as it circulates through the crust, mobilizing mainly metals and sulphides (Csotonyi *et al.*, 2006). Microbial communities present in and around these vents consist of thermophilic and hyperthermophilic bacteria and archaea, such as chemoautotrophic

sulphur reducer and oxidizers, and dissimilatory metal reducers (Barns and Nierzwicki-Bauer, 1997; Kashefi and Lovley, 2003; Csotonyi *et al.*, 2006). A study by Kashefi *et al.* (2001) demonstrated that hyperthermophilic dissimilatory Fe(III)-reducing bacteria (*Thermotoga maritime*) and archaea (*Pyrobaculum islandicum* and *Pyrococcoccus furiosus*) are able to extracellularly precipitate gold from gold(III) chloride at 100 °C under anoxic conditions in the presence of H_2 (Kashefi *et al.*, 2001). In this system, the precipitation of gold was an active, enzymatically catalysed reaction that depended on H_2 as the electron donor, and may involve a specific membrane-bound hydrogenase (Kashefi *et al.*, 2001). Anoxic conditions and abundant H_2 are common in hot springs, which occur where geothermally heated groundwater emerges from the Earth's crust. In Champagne Pool at Waitapu in New Zealand, the activity of thermophilic and hyperthermophilic bacteria and archaea led to the formation of gold- and silver-bearing sinters, with concentrations of these metals reaching more than 80 and 175 ng g^{-1} (material), respectively (Jones *et al.*, 2001); additionally, biomineralization of As and Sb bearing sulphide minerals was observed (Phoenix *et al.*, 2005). These studies suggest that microorganisms in deep subsurface and hydrothermal systems may directly contribute to the formation of primary gold mineralization.

Microbial mechanisms of gold solubilization

Microbially mediated gold solubilization depends on the ability of microorganisms to promote gold oxidation, and to excrete ligands capable of stabilizing the resulting gold ions by forming complexes or colloids. A number of microbial processes associated with different zones of the environment have been shown to fullfill these criteria. In arid, surficial environments (down to ~500 m below the land surface; Enders *et al.*, 2006) chemolithoautotrophic iron- and sulphur-oxidizing bacteria, such as *Acidothiobacillus ferrooxidans* and *A. thiooxidans*, and archaea, can form biofilms on metal sulphides (for example, gold-bearing pyrites and arsenopyrites), and obtain metabolic energy by oxidizing these minerals via a number of metabolic pathways, such as the sulphur oxidase pathway (*Sox*) and the reverse *Dsr* pathways (Nordstrom and Southam, 1997; Friedrich *et al.*, 2005; Southam and Saunders, 2005). Overall, the biofilms provide reaction spaces for sulphide oxidation, sulphuric acid for a proton hydrolysis attack and keep Fe(III) in the oxidized, reactive state (Sand *et al.*, 2001; Rawlings, 2002; Mielke *et al.*, 2003). The high concentrations of Fe^{3+} and protons then attack the valence bonds of the sulphides, which are degraded via the main intermediate thiosulphate (Sand *et al.*, 2001). The oxidation of sulphide minerals also leads to the release of associated metals into the environment (Southam and Saunders, 2005):

$$2FeAsS[Au] + 7O_2 + 2H_2O + H_2SO_4 \rightarrow Fe_2(SO_4)_3 + 2H_3AsO_4 + [Au] \quad (1)$$

In this process some iron and sulphur oxidizers (for example, *A. thioparus, A. ferrooxidans)* excrete thiosulphate, which in the presence of oxygen leads to gold oxidation and complexation (Aylmore and Muir, 2001):

$$Au + \tfrac{1}{4}O_2 + H^+ + 2S_2O_3^{2-} \rightarrow [Au(S_2O_3)_2^{3-}] + \tfrac{1}{2}H_2O \quad (2)$$

The solubilization of gold observed in a study of microcosms with quartz vein materials, containing sub-microscopic gold in arsenopyrite and pyrite from the Tomakin Park Gold Mine in temperate New South Wales, Australia, may have been mediated by this process. In biologically active systems (with live microbiota) a maximum of 550 ng gold per gram (d.w. material) was solubilized after 35 days of incubation. In contrast, sterile control microcosms displayed ten times lower concentration of solubilized gold that lay in the range of theoretical solubility for the system (McPhail *et al.*, 2006; Reith and McPhail, 2006).

Thiosulphate is also produced by other microbial processes involving different groups of microorganisms, and may promote gold solubilization in other environmental zones. SRB common in anoxic metal-rich sulphate-containing zones, for example, acid sulphate soils, form thiosulphate during the reduction of sulphite with H_2 and formate (Fitz and Cypionka, 1990). *Streptomycetes fradiae*, a common soil actinomycete, produces thiosulphate as a result of the metabolization of sulphur from cystine (Kunert and Stransky, 1988).

In a microcosm study with organic matter-rich auriferous (gold-containing) soils from the Tomakin Mine Park Mine in New South Wales, Australia, the biologically active microcosm displayed up to 80 wt.% gold solubilization within 20–45 days of incubation, after which it was re-adsorbed to the solid soil fractions (Figure 2; Reith and McPhail, 2006). In biologically active microcosms amended with native 99.99% pure gold pellets, gold was liberated from the pellets as well as the soil, and the formation of bacterial biofilms on the gold pellets was observed; in contrast, gold was not solubilized in sterile controls (Figure 2; Reith and McPhail, 2006). Similar results were also obtained in microcosms with auriferous soils from semi-arid and tropical zones in Australia (Reith and McPhail, 2007). The solubilization of gold in the microcosms appeared to be linked to the activity of heterotrophic bacteria that dominate the bacterial communities in organic matter-rich soils. A mechanism for gold solubilization involving heterotrophic bacteria (that

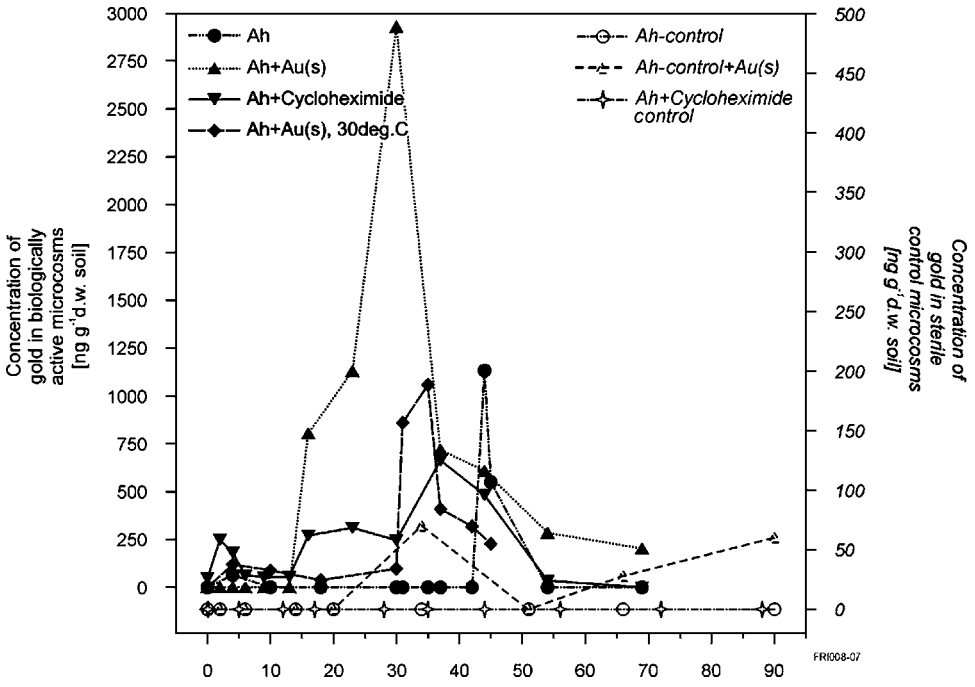

Figure 2 Concentration of solubilized gold in the solution in slurry microcosms (1 : 4 w/w soil to water) with Ah-horizon soil from the Tomakin Park Gold Mine in New South Wales, Australia, incubated biologically active or inactive (sterilized) for up to 90 days at 25 (diagram adapted from Reith and McPhail, 2006).

is, *Bacillus subtilis*, *B. alvei*, *B. megaterium*, *B. mesentericus, Serratia marcescens*, *Pseudomonas fluorescens*, *P. liquefaciens* and *Bacterium nitrificans*) has been described in earlier studies, where under *in vitro* conditions up to 35 mg l^{-1} (medium) of gold was solubilized as gold–amino acid complexes (Lyalikova and Mockeicheva, 1969; Korobushkina *et al.*, 1974, 1976; Boyle, 1979; Korobushkina *et al.*, 1983). DNA fingerprinting and assessment of the metabolic function of the bacterial community during the incubation of the microcosms, combined with gold and amino-acid analyses of the solution phase, indicated that the bacterial community changed from a carbohydrate- to amino acid-utilizing population concurrently with the solubilization and re-precipitation (Reith and McPhail, 2006). The bacterial community in the early stages of incubation (0–30 days) produced an overall excess of free amino acids, and up to 64.2 μM of free amino acids were measured in the soil solution within the first 30 days of incubation (Reith and McPhail, 2006). The bacterial community in the later stages of incubation (after 40–50 days) presumably metabolized these gold-complexing ligands (the concentration of free amino acids was reduced to ~8.0 μM), and thus destabilized gold in solution, which led to the re-adsorption of gold to the solid soil fractions, and in particular the organic fraction (Reith and McPhail, 2006). These results suggest a link between gold solubilization and microbial turnover of amino acids in auriferous soils.

Another possible microbial mechanism for gold solubilization in auriferous soils is the oxidation and complexation of gold with cyanide (excreted by cyanogenic microbiota) leading to the formation of dicyanoaurate complexes ($Au(CN)_2^-$; Rodgers and Knowles, 1978; Saupe *et al.*, 1982; Faramarzi *et al.*, 2004; Faramarzi and Brandl, 2006):

$$Au + 2CN^- + \tfrac{1}{2}O_2 + H_2O \rightarrow [Au(CN)_2]^- + 2OH^- \quad (3)$$

At physiological pH, cyanide is mainly present as HCN and therefore volatile (Faramarzi and Brandl, 2006). However, in the presence of salts and cyanicidic compounds, for example metal ions, volatility is reduced, and thus cyanide produced by bacteria may directly affect gold solubilization in the environment (Faramarzi and Brandl, 2006). An *in vitro* study using the cyanogenic bacterium *Chromobacterium violaceum* demonstrated that biofilms grown on gold-covered glass slides were able to solubilize 100 wt% of gold within 17 days, with concentrations of gold and free cyanide in solution reaching 35 and 14.4 mg l^{-1} (medium), respectively (Campbell *et al.*, 2001). Similar results were also obtained when *C. violaceum* was incubated with biooxidized gold ore concentrate; here up to 0.34 and 9 mg l^{-1} (medium) of gold and cyanide, respectively, were detected in solution within 10 days (Campbell *et al.*, 2001). *Pseudomonas plecoglossicida* has been shown to solubilize 69% of gold from shredded printed circuit boards after 80 h of incubation, by producing up to 500 mg [$Au(CN)_2^-$] per litre (medium; Faramarzi and Brandl, 2006).

Cyanide production and excretion in soils has commonly been ascribed to higher plants and fungi, but is also widespread in soil bacteria, such as

P. fluorescens, *P. aeruginosa*, *P. putida*, *P. syringae* and *B. megaterium,* which produce cyanide via the membrane-bound enzyme complex, HCN synthase (Knowles, 1976; Wissing and Andersen, 1981; Faramarzi *et al.*, 2004; Faramarzi and Brandl, 2006). Cyanide has no apparent function in primary metabolism, is optimally produced during growth limitation and may offer the producer, which is usually cyanide tolerant, a selective advantage by inducing cyanide toxicity in other organisms, thus it can be classified as a typical secondary metabolite (Castric, 1975). Glycine is a common metabolic precursor for the microbial production of cyanide (Knowles, 1976; Rodgers and Knowles, 1978). The highest concentration of free glycine in solution in Tomakin Park Gold Mine soil microcosms was detected after 20 days of incubation (Reith and McPhail, 2006), and cyanide concentration up to $0.36\,mg\,l^{-1}$ (soil solution) was measured in solution in recent Tomakin soil microcosms (F Reith, personal communication), suggesting that the solubilization of gold as dicyanoaurate complex, may occur in addition to gold solubilization with amino acids.

Overall, microbially mediated gold solubilization may occur as a consequence of the microbial production and excretion of a number of gold-complexing metabolites, that is, thiosulphate, amino acids and cyanide, in the presence of oxygen. Gold solubilization via the thiosulphate mechanism is expected in organic carbon matter-poor environments, where chemolithoautotrophic processes and populations dominate, for example, primary sulphide mineralization. Gold solubilization via the amino acid and/or cyanide mechanisms may occur in organic matter-rich top and rhizosphere soils, where plant root exudates may directly lead to gold solubilization. Root exudates also provide nutrients for resident microbiota, such as cyanogenic fungi and bacteria (for example, *Pseudomonas* sp.), which may further increase gold solubilization (Bakker and Schippers, 1987). These plant–microbe interactions in the rhizosphere may also lead to an increased uptake of gold by plants and/or contribute to the general dispersion of gold in the environment (Khan, 2005).

Bioaccumulation of gold by microorganisms

Laboratory studies have been conducted to elucidate the interactions of gold with microorganisms using gold(III) chloride- (Beveridge and Murray, 1976; Darnall *et al.*, 1986; Greene *et al.*, 1986; Watkins *et al.*, 1987; Gee and Dudeney, 1988; Karamushka *et al.*, 1990a, b; Dyer *et al.*, 1994; Southam and Beveridge, 1994, 1996; Canizal *et al.*, 2001; Kashefi *et al.*, 2001; Karthikeyan and Beveridge, 2002; Nair and Pradeep, 2002; Ahmad *et al.*, 2003; Nakajima, 2003; Tsuruta, 2004; Keim and Farina, 2005; Lengke *et al.*, 2006a, b, c, 2007; Reith *et al.*, 2006), gold(I) thiosulphate- (Lengke and Southam, 2005, 2006, 2007; Lengke *et al.*, 2006a), gold L-asparagine- (Southam *et al.*, 2000), aurothiomalate-complexes (Higham *et al.*, 1986) and colloidal gold-containing solutions (Karamushka *et al.*, 1987b, 1990b; Ulberg *et al.*, 1992). In study with 30 different microorganisms (eight bacteria, nine actinomycetes, eight fungi and five yeasts, Nakajima (2003) found that the bacteria were more efficient in removing gold(I/III) complexes from solution than other groups of microorganisms. These results were confirmed by Tsuruta (2004), who studied gold(III) chloride bioaccumulation in 75 different species (25 bacteria, 19 actinomycetes, 17 fungi and 14 yeasts), and found that gram-negative bacteria, such as *Acinetobacter calcoaceticus* and *P. aeruginosa*, had the highest ability to accumulate gold. Some bacteria also appear to be able to selectively and/or actively precipitate gold complexes, which suggests that gold bioaccumulation in these organisms may be part of a metabolic process, rather than passive biosorption that is often observed in fungi and algae (Savvaidis *et al.*, 1998; Khoo and Ting, 2001; Nakajima, 2003; Figure 3). Some studies also point to different active, bioaccumulation mechanisms depending on whether the gold is extracellularly (that is, along the plasma or outer membranes; Figures 3a–c) or intracellularly (that is, in the cytoplasm, Figure 3d) precipitated, or accumulated in minerals or exopolymeric substances (Higham *et al.*, 1986; Southam *et al.*, 2000; Ahmad *et al.*, 2003; Konishi *et al.*, 2006; Lengke and Southam, 2006, 2007; Lengke *et al.*, 2006a, b, c, 2007; Reith *et al.*, 2006). These mechanisms may occur individually or in combination in different bacterial species (Higham *et al.*, 1986; Lengke and Southam, 2006, 2007).

In the presence of H_2 as electron donor the anaerobic Fe-reducing bacterium *Shewanella algae* has been shown to intracellularly precipitate metallic gold nanoparticles (Konishi *et al.*, 2006). Intracellular gold precipitation was also shown in the cyanobacterium *Plectonema boryanum* (Lengke *et al.*, 2006a, b, c; Figure 3d), and an alkalotolerant actinomycete (*Rhodococcus* sp.; Ahmad *et al.*, 2003). The mechanisms of gold bioaccumulation by *P. boryanum* from gold(III) chloride solutions have been studied using XAS (Lengke *et al.*, 2006c). The results show that the reduction mechanism of gold(III) chloride to metallic gold by the cyanobacterium involves the formation of an intermediate gold(I) species, similar to a gold(I) sulphide (Lengke *et al.*, 2006c). The sulphur presumably originates from cysteine or methionine in cyanobacterial proteins or binding to glutathione (Fahey *et al.*, 1978). *Sporasarcina ureae* was able to grow in the presence of up to 10 p.p.m. of gold complexed by L-asparagine, but not in the presence of gold(III) chloride (Southam *et al.*, 2000). During growth with gold-L-asparagine, *S. ureae* did not precipitate

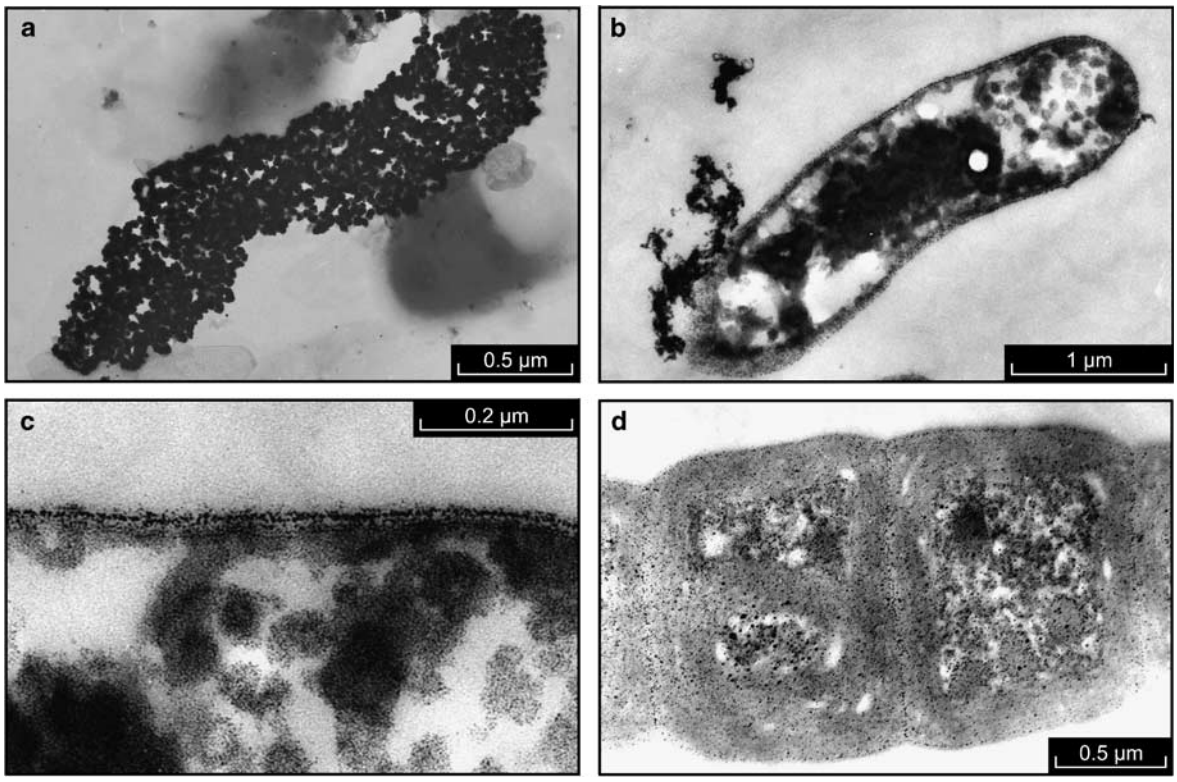

Figure 3 TEM micrographs of sulphate reducing (**a**), thiosulphate oxidising (**b**, **c**) and cyanobacteria (**d**) incubated in the presence of gold(I/III) complexes. (**a**) *Desulfovibrio* sp. encrusted with gold, as indicated by dark particles (after 148 days incubation at 25 °C with 500 p.p.m. gold as $[Au(S_2O_3)_2^{3-}]$); (**b**, **c**) Thin sections of *A. thiooxidans* cells with nanoparticles of gold deposited along the outer wall layer, periplasm and cytoplasmic membrane (after 75 days incubation at 25 °C with 50 p.p.m. of gold as $[Au(S_2O_3)_2^{3-}]$); (**d**) Thin section of *P. boryanum* showing nanoparticles of gold deposited inside cells (after 28 days incubation at 25 °C with 500 p.p.m. of gold as $[AuCl_4^-]$).

metallic gold intracellularly; the intracellular immobilization was associated with the low molecular weight protein fraction, which appeared to regulate the detoxification (Southam *et al.*, 2000). This suggests that the intracellular formation of gold nanoparticles may occur via an intermediate gold(I)–protein complex; the reduction of the intermediate complex to metallic gold may then be regulated by its concentration in the cell.

The intracellular precipitation and formation of gold nanoparticles (<10 nm) from gold(I) thiosulphate was also observed in SRB (that is, *Desulfovibrio* sp.; Lengke and Southam, 2006, 2007). In addition, the presence of localized reducing conditions produced by the bacterial electron transport chain via energy-generating reactions led to extracellular precipitation of gold, and the formation of hydrogen sulphide triggered iron sulphide formation, which caused removal of gold from solution by adsorption and reduction on iron sulphide surfaces (Lengke and Southam, 2006, 2007; Figure 3a). In earlier studies with *B. subtilis*, Beveridge and Murray (1976) have shown that the extracellular reduction and precipitation of gold from gold(III) chloride solution were selective, as similar processes did not occur for Ag(I). In solutions containing a combination of gold(III) chloride, Cu^{2+}, Fe^{2+} and Zn^{2+}, gold was selectively adsorbed by *B. subtilis* (Gee and Dudeney, 1988) and *Spirulina platensis* (Savvaidis *et al.*, 1998). The extracellular accumulation of gold(III) chloride and colloidal gold in *Bacillus* sp. was further investigated by Ulberg *et al.* (1992) and Karamushka *et al.* (1987a, b), who found that bioaccumulation of gold by *Bacillus* sp. depended on the chemical fine structure of the cell envelope, and involved functional groups of proteins and carbohydrates bound in the plasma membrane. In further studies, the accumulation of gold by *Bacillus* sp. was shown to be directly dependent on the metabolic activity of the cells, and particularly the hydrolysis of ATP (adenosine triphosphate) by the enzyme ATPase in the plasma membrane (Karamushka *et al.*, 1990a, b; Ulberg *et al.*, 1992).

Gold accumulation in an exopolysaccharide capsule was observed in *Hyphomonas adhaerens*; cultures that were able to form the capsule-precipitated gold from gold(III) chloride solutions, whereas a mutant without capsule was not able to do so (Quintero *et al.*, 2001). Gold accumulation in exopolymeric substances also played a role in the detoxification of gold(III) chloride by *P. aeruginosa*, which displayed a four times higher viability when grown as a biofilm (containing exopolymeric substances (EPS)) compared to free planktonic cells when subjected to 0.1 mM gold(III) chloride

(Karthikeyan and Beveridge, 2002). *Burkholderia cepecia* has been shown to grow in the presence of 0.5–5 mM concentrations of gold(I) thiolates, such as aurothiomalate, using a number of apparent adaptation mechanisms (Higham *et al.*, 1986). As a result, the size of cells increased, cells formed polyhydroxybutyrate granules, accumulated gold and excreted a low molecular weight protein called thiorin, which may have bound the gold complexes in the culture medium (Higham *et al.*, 1986).

To summarize this section, bacteria are particularly efficient in accumulating gold complexes, and have developed a number of mechanisms that enable them to accumulate gold intra- or extracellularly, or in products of their metabolism, suggesting that this may lead to advantages for the survival of these bacterial populations.

Advantages of active gold bioaccumulation

The capability to actively accumulate gold using enzyme systems to deal with gold toxic complexes may result in advantages for the survival of microbial populations by being able to (1) detoxify the immediate cell environment, (2) use gold-complexing ligands to gain metabolic energy or (3) use gold as a micronutrient.

The mechanisms of gold toxicity in bacterial cells are little understood, but it is likely that gold complexes cause toxicity and cell death by disrupting the normal redox activity of cell membranes, and by affecting the permeability of cell walls and membranes by cleaving disulphide bonds in peptides and proteins (Witkiewicz and Shaw, 1981; Carotti *et al.*, 2000; Karthikeyan and Beveridge, 2002). It is clear, however, that gold complexes are toxic to microorganisms at very low concentration, the minimal inhibitory concentration (MIC) of gold(III) chloride in *Escherichia coli* is 20 μM (~4 p.p.m.) and toxic effects to the organisms start at approximately 1/1000 of the MIC (Nies, 1999). Gold concentrations in soil solutions from auriferous soils can reach more than 100 p.p.b. (McPhail *et al.*, 2006; Reith and McPhail, 2006), and are possibly even higher in solutions surrounding gold nuggets. Thus, toxic effects to microorganisms in these zones are likely to occur, and having developed mechanisms to detoxify the immediate cell environment would certainly mean a survival advantage.

For detoxification processes leading to the reductive precipitation of gold to occur, microorganisms need to able to detect, and subsequently reduce gold complexes. In bacteria, cytoplasmic metal ion-responsive transcriptional regulators are important in regulating the expression of genes involved in metal ion homoeostasis and efflux systems (Silver, 1996; Nies, 1999, 2003; Hobman, 2007). The *MerR* family of transcriptional activators are metal-sensing regulators that are found in a variety of bacteria and have a common design, but have evolved to recognize and respond to different metals (Silver, 1996; Nies, 1999, 2003; Hobman, 2007). In a study with *E. coli*, Stoyanov and Brown (2003) demonstrated for the first time the specific regulation of transcription by gold complexes. They showed that *CueR*, an *MerR*-like transcriptional activator that usually responds to Cu^{+}, is also activated by gold complexes (presumably gold(III) chloride), and that this activation is promoted by specific binding of gold to the cysteine residues 112 and 120 (Cys 112 and 120). In a recent study Checa *et al.* (2007) characterized a new transcriptional regulator (*GolS*) in the bacterium *Salmonella enterica*. *GolS* shares with other *MerR* family regulators the metal-binding cysteine residues Cys112 and 120, but retains exclusive specificity to gold(III) chloride. The presence of at least two open reading frames whose expression is activated by *GolS*, one of which is a predicted transmembrane efflux ATPase (*GolT*), and the other a predicted metallochaperone (*GolB*), suggests a mechanism for resistance to gold complexes that is consistent with the organization of many metal resistances, where an *MerR* regulator controls both its own production and that of a P-type ATPase and a chaperone protein (Checa *et al.*, 2007; Hobman, 2007).

The *MerR* family of regulators is also important in regulating metal resistance in *C. metallidurans*, with Rmet_3523, an apparent ortholog to *GoIS*, present in its genome (Nies and Silver, 1995; Mergeay *et al.*, 2003; Socini FC, personal communication). *C. metallidurans* is associated with secondary gold grains, actively reduces gold complexes and accumulates native gold, and thus may provide a critical link between the precipitation of gold observed in the laboratory and the observed 'bacterioform' gold in natural systems (below; Reith *et al.*, 2006). *C. metallidurans* is a gram-negative facultative chemolithoautotrophic β-*proteobacterium*, that is able to withstand high concentrations of heavy metal ions (for example, Cu, Pb, Zn, Cd, Ag and Au; Mergeay *et al.*, 2003; Reith *et al.*, 2006). Its extraordinary heavy metal resistance and ability to accumulate metals on its surface promoting detoxification hail from multiple layers of P-type ATPase efflux pumps activated by *MerR*-like regulator genes (Legatzki *et al.*, 2003; Mergeay *et al.*, 2003; Nies, 2003). A recent study of *C. metallidurans* strain CH34 has identified eight putative metal-transporting ATPases involved in metal binding compared with the average of three P1-ATPases in other microorganisms (Monchy *et al.*, 2006).

Other gram-negative bacteria, such as *P. boryanum*, produce membrane vesicles that may act as protective shields against toxic metals (Silver, 1996). When *P. boryanum* faces high concentrations of $[Au(S_2O_3)_2^{3-}]$, the cells release membrane vesicles (Lengke *et al.*, 2006a). These vesicles remain associated with the cell envelope, 'coating' the cells,

which help prevent uptake of $[Au(S_2O_3)_2^{3-}]$, keeping it away from sensitive cellular components. The interaction of $[Au(S_2O_3)_2^{3-}]$ with the vesicle components causes the precipitation of elemental gold on the surfaces of the vesicles, possibly through the interaction with phosphorus, sulphur or nitrogen ligands from the vesicle components (Lengke *et al.*, 2006a). Thus, bacteria like *S. enterica*, *C. metallidurans and P. boryanum* appear to have evolved effective mechanisms to detect and avoid gold toxicity, which will allow them to thrive in gold-rich environments.

The precipitation of gold from gold(I) thiosulphate solutions has been observed in the presence of thiosulphate-oxidizing bacteria (Figures 3b and c; *A. thiooxidans*; Lengke and Southam, 2005). The gold precipitated by *A. thiooxidans* was accumulated inside the bacterial cells as fine-grained colloids ranging between 5 and 10 nm in diameter and in the bulk fluid phase as crystalline micrometre-scale gold (Lengke and Southam, 2005). While gold was deposited throughout the cell, it was concentrated along the cytoplasmic membrane, suggesting that gold precipitation was likely enhanced via electron transport processes associated with energy generation (Figures 3b and c; Figure 4). When gold(I) thiosulphate entered the cells as a complex, thiosulphate was used in a metabolic process within the periplasmic space, and gold(I) was transported into the cytoplasm by the chemiosmotic gradient across the cytoplasmic membrane and/or through ATP hydrolysis. Since gold(I) cannot be degraded, gold(I) will form a new complex or be reduced to metallic gold(0). Metallic gold is potentially re-oxidized, forming a new complex, which can also be transported outside the cytoplasm (Figure 4).

Gold is believed to be non-essential for microbial nutrition (Madigan and Martinko, 2006). However, in the presence of gold, *Micrococcus luteus* has been shown to oxidize methane more efficiently by forming a nicotinamide adenine dinucleotide oxidase that includes a gold(I/III)-redox couple in its active centre (Levchenko *et al.*, 2000, 2002). This redox complex appears to activate methane by forming a CH_3–gold(III) complex similar to methane-monooxygenase commonly used by methanotrophs (Levchenko *et al.*, 2000). This may represent an alternative way for bacteria to utilize methane, but it is not known if other methanotrophic bacteria are able to form similar gold-containing enzymes, or if other gold-containing enzymes exist in any other microorganisms. However, in a recent study methanotrophs have been detected on secondary gold grains from an Australian mine, suggesting an environmental association of methane-oxidizing bacteria with gold (Reith, 2005).

To summarize, the general detoxification of gold complexes by bacteria is likely controlled by *MerR*-like regulators, which encode their own production, and that of P-type ATPases and chaperone proteins that transport gold out of the cells. Furthermore *A. ferrooxidans* has been shown to be able to utilize the gold thiosulphate complexes for energy generation, and a gold-containing enzyme involved in methane oxidation has been described in *M. luteus*.

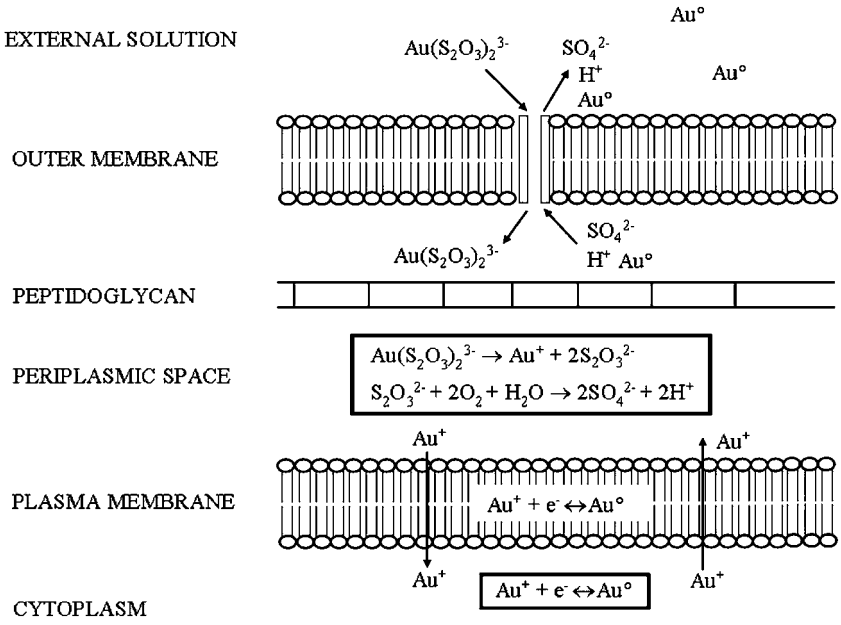

Figure 4 Schematic model for $[Au(S_2O_3)_2^{3-}]$ utilization and gold precipitation by *A. thiooxidans* (adapted from Lengke and Southam, 2005). When $[Au(S_2O_3)_2^{3-}]$ is the only available energy source a pore in the outer membrane allows the exchange of sulphur species and gold. Sulphur reactions occur in the periplasmic space, while gold reduction process may occur in the cytoplasm. The oxidized sulphur and reduced gold are released as waste products through the outer membrane pore, which is a possible origin for gold observed inside and at bacterial cells.

Microbially mediated formation of secondary gold

The origin of coarse gold grains and nuggets has long been the subject of discussion among geologists studying placer deposits (Figure 5a). Three models have been established to explain their formation: detrital origin, chemical accretion and a combination of both (Boyle, 1979). One major problem is that gold grains in supergene environments are commonly coarser grained, that is, larger, than that observed in potential source rocks (Wilson, 1984; Watterson, 1992; Mossman *et al.*, 1999). A second problem is the wide range of morphologies associated with secondary gold, which are not commonly observed in source ore deposits. These morphologies include wire, dendritic, octahedral, porous and sponge gold (Figure 5b; Kampf and Keller, 1982; Leicht, 1982; Lieber, 1982; Wilson, 1984; Watterson, 1992; Márquez-Zavalía *et al.*, 2004). Hence, many authors have provided field-based evidence that secondary processes are responsible for their formation that occur mainly in soils or shallow regolith (Webster and Mann, 1984; Wilson, 1984; Clough and Craw, 1989; Watterson, 1992; Craw and Youngson, 1993; Lawrance and Griffin, 1994). Previous sections have shown that microbial gold solubilization and precipitation may occur in surficial environments. However, the extent and rate at which these secondary processes drive gold biomineralization, as opposed to purely abiotic secondary processes, is not clear.

Possible bacterial textures in secondary gold

Watterson (1992) first reported structures that resembled gold-encrusted microfossils on placer gold specimens from Lillian Creek in Alaska, and postulated a biological mechanism for the formation of gold grains. After studying 18 000 grains from different sites in Alaska and observing similar lace-like networks of micrometre-size filiform gold on the majority of these grains, he concluded that these gold grains were of bacterial origin (Watterson, 1992). He interpreted the observed structures as pseudomorphs of a *Pedomicrobium*-like budding organism. However, after producing analogous structures abiotically with natural and artificial gold amalgams using hot nitric acid dissolution, Watterson and others concluded that the observed morphologies alone could not be considered adequate evidence of microbial origin of these gold grains (Watterson, 1994; note, this geochemically extreme condition will not occur in placer environments).

Similar textures were detected on a wide variety of placer gold specimens that have not been subjected to any chemical treatment. Numerous specimens from different locations in Australia, South America and Canada have been studied, and many of the crevices in gold grains were covered by *Pedomicrobium*-like pseudomorphs similar to those described from the Alaskan specimens (Figures 5e and f; Bischoff *et al.*, 1992; Mann, 1992; Keeling, 1993; Bischoff, 1994, 1997; Reith, 2005; Reith *et al.*, 2006). Bud-like gold growths are common on the surfaces of authigenic gold from low-redox placer settings in southern New Zealand (Figures 5g and h; Falconer *et al.*, 2006). These latter examples show a range of textures in a transition, with increasing mineralization, from isolated buds to coalesced buds to chains of buds and ultimately to infilled cavities (Falconer *et al.*, 2006). Despite the apparent similarity of these buds to *Pedomicrobium*-like pseudomorphs, no direct connection between gold and bacteria had been established in the above examples.

Direct associations between secondary gold and bacteria

A recent study of untreated secondary gold grains from two field sites has provided this important link; using SEM and CSLM combined with nucleic acid staining, bacterial pseudomorphs and active bacterial biofilms, respectively, were revealed on the surfaces of these grains (Figures 5c and d; Reith *et al.*, 2006). Molecular profiling showed that unique, site-specific bacterial communities are associated with gold grains that differ from those dominating the surrounding soils. 16S rDNA clones belonging to the genus *Ralstonia* and bearing a 99% similarity to *C. metallidurans* were present on all DNA-positive gold grains, but were not detected in the surrounding soils (Reith *et al.*, 2006). The ability of *C. metallidurans* to actively accumulate gold from solution was successfully tested suggesting that *C. metallidurans* may contribute to the formation of secondary gold grains (Reith *et al.*, 2006).

Octahedral plate-like secondary gold platelets are common in oxidized zones surrounding primary deposits (Figure 5b; Wilson, 1984; Lawrance and Griffin, 1994; Gray, 1998). Southam and Beveridge (1994, 1996) demonstrated the formation of metallic gold with octahedral habit by organic phosphate and sulphur compounds derived from bacteria at pH $\sim$2.6. The formation of metallic gold in phosphorus-, sulphur- and iron-containing granules within magnetotactic cocci has also been observed (Keim and Farina, 2005). Lengke *et al.* (2006a, b, c) observed octahedral gold, formed by *P. boryanum* at pH 1.9–2.2 and at 25–200 °C (Figure 5b). The bacteria, which were killed in this process, were able to intracellularily immobilize more than 100 $\mu g\,mg^{-1}$ (d.w. bacteria; Figure 3d). In these systems, bacterial autolysis was initialized, proteins were released and pseudo-crystalline gold was formed, which was transformed into crystalline octahedral gold (up to 20 μM; Southam and Beveridge, 1994, 1996; Lengke *et al.*, 2006a, b). The ability of sulphur-oxidizing (Lengke and Southam, 2005) and -reducing (Lengke and Southam, 2006) bacteria to transform gold

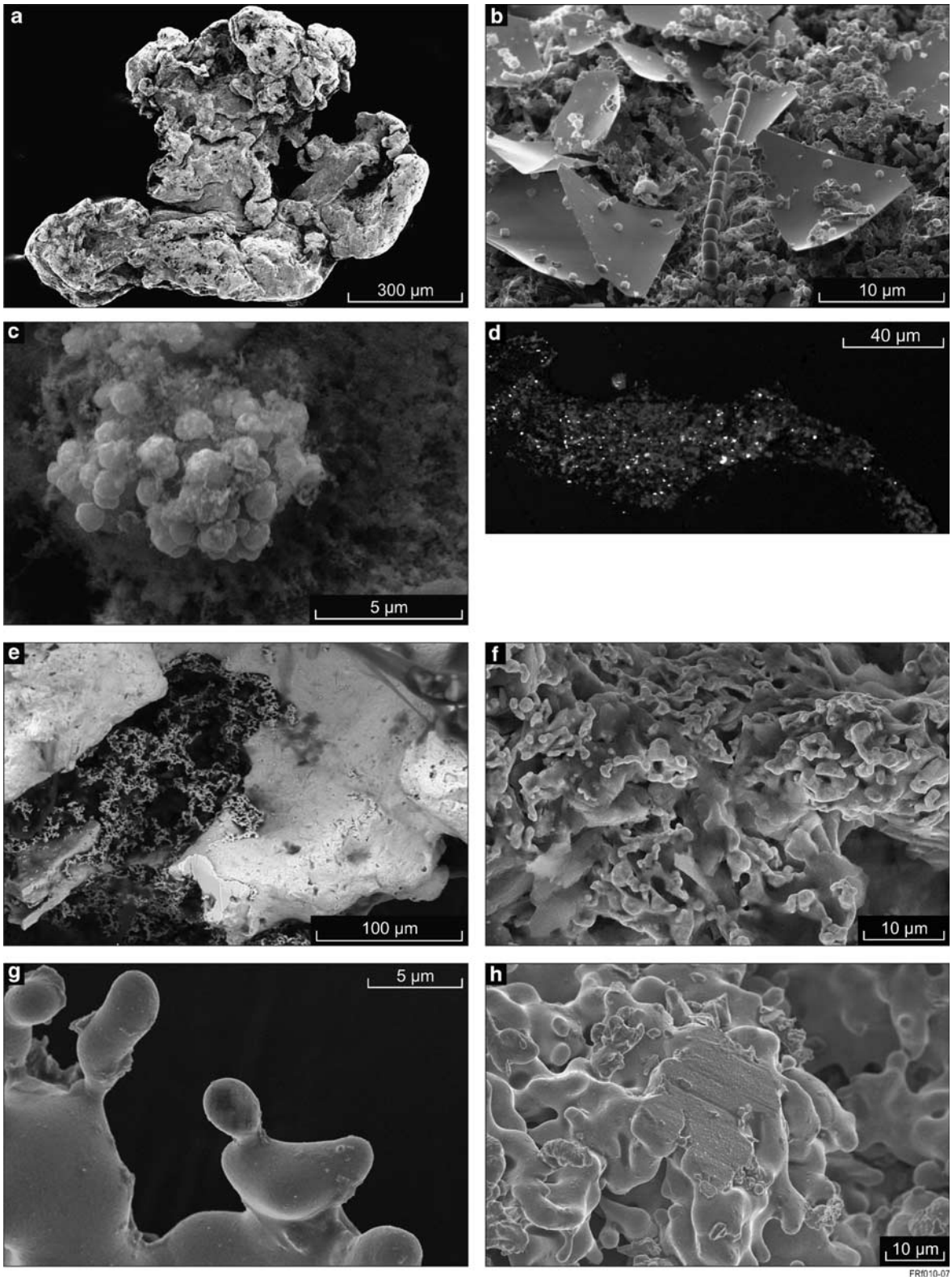

Figure 5 Bioaccumulation of gold by microorganisms may lead to biomineralization of secondary gold. (**a**) An SEM micrograph of a secondary gold grain from the Tomakin Park Gold Mine (New South Wales, Australia); (**b**) SEM micrograph of octahedral gold platelets from cyanobacteria–$AuCl_4^-$ experiments (after 28 days incubation at 25 °C with 500 p.p.m. of gold as [$AuCl_4^-$]); (**c**) SEM micrograph of a biofilm growing on a gold pellet incubated for 70 days in a biologically active slurry microcosms with soil from the Tomakin Park Gold Mine; (**d**) confocal microscopic images of a fluorescently stained (DAPI) biofilm on a secondary gold grain from the Hit or Miss Gold Mine (Queensland, Australia); (**e**, **f**) surface depression of gold grain extensively covered with a three-dimensional network of 'bacteriofrom' gold on a gold grain from the Hit or Miss Gold Mine; (**g**, **h**) detail of a rounded and budding cell pseudomorph forming 'bacterioform' gold from the Waimumu–Waikaka Quartz Gravels, New Zealand (adapted from Falconer *et al.*, 2006).

complexes into octahedral gold, suggests that bacteria contribute to the formation of gold platelets in the supergene environment (Figure 5b).

Secondary gold in quartz pebble conglomerates

Quartz pebble conglomerates are mature fluvial sedimentary rocks that commonly contain placer gold. The most famous auriferous QPC is the Archaean Witwatersrand sequence of South Africa (Frimmel *et al.*, 1993; Minter *et al.*, 1993), but similar younger deposits occur in California and New Zealand (Falconer *et al.*, 2006; Youngson *et al.*, 2006). Most QPC sequences contain detrital and authigenic sulphide minerals (Minter *et al.*, 1993; Falconer *et al.*, 2006). Authigenic sulphide and gold textures are well preserved in New Zealand QPCs, and also in the regionally metamorphosed Witwatersrand (Falconer *et al.*, 2006). Bud-like authigenic gold morphologies that occur within New Zealand QPCs are similar in appearance to 'filamentous' gold that occurs in the Witwatersrand (Falconer *et al.*, 2006). Notably, both QPC contain gold as an Au–Ag–Hg alloy and it is speculated that mercury from the Au–Ag–Hg alloy may play a significant role in the development of such 'bacterioform' textures (Falconer *et al.*, 2006). In addition, a wide range of other authigenic gold textures occur, including octahedral and 'triangular' (pseudotrigonal octahedral) crystals and porous sheets (Clough and Craw, 1989; Falconer *et al.*, 2006).

There is commonly a close association between secondary gold and organic matter in QPC sequences (Mossman *et al.*, 1999; Falconer *et al.*, 2006). This organic matter could have provided a suitable environment for bacteria to thrive, although no direct link has yet been established between authigenic gold and bacteria in these deposits. The carbonaceous matter, including solidified bitumen, associated with much of the gold in the Witwatersrand basin is thought to be of cyanobacterial origin primarily based on its isotopic composition (Hoefs and Schidlowski, 1967); however, it also possesses more contentious structural evidence of its bacterial origin due to the presence of filaments, which that are the size and shape of modern filamentous cyanobacteria (Dyer *et al.*, 1984, 1994; Mossman and Dyer, 1985; Mossman *et al.*, 1999). Alternatively, Spangenberg and Frimmel (2001) suggested that these filaments were introduced as liquid hydrocarbons, that is, diagenetic products of microbial origin. The occurrence of octahedral gold and diagenetic carbon suggests that these microbial products are also able to catalyse the formation of crystalline gold.

Conclusions and future directions

This review has shown that microorganisms are capable of actively solubilizing and precipitating gold, and suggests that they may play a key role in the dispersion and concentration of gold under surface conditions, in the deep subsurface and in hydrothermal zones (Figure 1). Results from recent studies provide experimental evidence for a number of genetic, biochemical and physiological mechanisms that microbes may use to drive biogeochemical gold cycling (Figure 1). These studies indicate that microorganisms (1) mediate gold solubilization via excretion of a number of metabolites (for example, thiosulphate, amino acids and cyanide); (2) have developed direct genomic and biochemical responses to deal with toxic gold complexes and (3) are able to precipitate gold intra- and extracellularly, and in products of their metabolism (for example, EPS and sulphide minerals). Bacteria such as *S. enterica* and *C. metallidurans* apparently use *MerR*-type gold-specific transcriptional regulators, which directly control detoxification via P-type ATPase efflux pumps and metal chaperone proteins. *A. ferrooooxidans* gains metabolic energy by oxidizing thiosulphate from gold(I) thiosulphate complexes, and a gold-containing enzyme has been shown to improve methane oxidation in *M. luteus*. Bioaccumulation processes in *C. metallidurans* are possibly linked to the biomineralization of secondary 'bacterioform' gold. Sulphur-oxidizing and -reducing bacteria and cyanobacteria may be linked to the formation of secondary octahedral gold, and may have contributed to the formation of quartz pebble placer deposits.

While it is likely that many environmentally relevant groups of microbes may be involved in the biogeochemical cycling of gold, further experimental evidence for the proposed mechanisms needs to be obtained. The kinetics of gold-affecting processes in the environmental systems are little understood with respect to current environmental processes, and even less if geological times scales are taken into account. Furthermore, little is known about genomic and biochemical pathways leading to gold toxicity and detoxification in environmentally relevant organisms, nor has gold turnover been successfully linked to structures and activities of complex microbial communities in mineralized zones and auriferous soils. Thus, studies using genomic and proteomic approaches, such as RNA- and protein-expression arrays and quantitative PCR, have to be conducted to understand the biochemistry of gold turnover in different bacteria. Metagenomic and proteomic approaches in combination with field studies, microcosms and culturing studies may provide ways to link microbial community structures and activities with the turnover of gold in natural systems. Further synchrotron radiation-based experiments, such as μXRF, μXANES and μEFAXS of gold in individual cells need to be conducted to assess its distribution, speciation and association in cells. These studies may thus establish a direct link between the biogeochemical cycling of gold and microbial community activities,

and provide a model for the assessment of other potential biogeochemical metal cycles, for example, Ag, Pt and Pd.

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