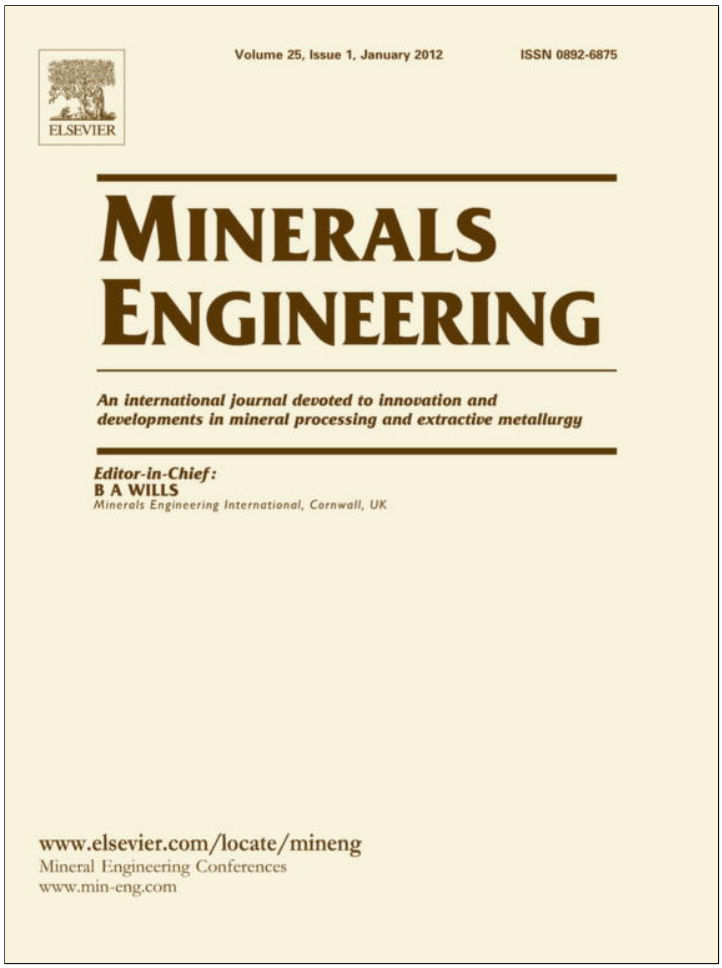

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The future of biotechnology for gold exploration and processing

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ABSTRACT

The role of biological agents in the mining industry is currently limited to the use of microorganisms in bioleaching and bioremediation. However, there are a number of ways in which biotechnology will be used in the near future to aid the mining industry. This review focuses on the development of novel biotechnologies and the role they will play in gold exploration, processing and remediation. The development of these biotechnologies has been enabled by advances in our molecular-level understanding of the role microorganisms play in the solubilisation, dispersion and precipitation of gold, brought upon by the rapid development of molecular genetic techniques over the past decade. This fundamental knowledge is now being used to develop new methods for gold exploration, processing and remediation. An understanding of the distribution of microbial species in soils overlying mineralisation can be utilised to develop bioindicator systems that assist with gold exploration. An in-depth knowledge of how microorganisms interact with gold complexes is being used to develop biosensors, further supporting exploration. Processing technologies are being improved based upon advances in our understanding of the interactions between microorganisms, cyanide and gold. For instance, cyanide-producing microorganisms are being investigated for use *in situ* leaching of gold. In turn, the use of cyanide-utilising microorganisms for the degradation of cyanide is being explored. Combined the implementation of biotechnologies in the gold mining sector is set to revolutionise the industry, leading to the greener, more efficient extraction of gold.

Contents

Abbreviations: CDRR, coupled dissolution replacement reaction; DGGE, denaturing gradient gel electrophoresis; EBSD, electron back-scattered diffraction; FIB, focused ion beam; FIB-SEM, focused ion beam-scanning electron microscopy; HR-TEM, high resolution-transmission electron microscopy; MIC, minimal inhibitory concentration; LA-ICP-MS, laser-ablation inductively-coupled mass spectroscopy; TEM, transmission electron microscopy; TGGE, thermal gradient gel electrophoresis; T-RFLP, terminal-restriction fragment length polymorphism; RISC, reduced inorganic sulphur compounds; ROM, run-of-mine; SSCP, single strand conformation polymorphism; VMS, volcanogenic massive sulphide; μ-XRF, μ-X-ray fluorescence.

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1. Introduction

While the value of key minerals, such as nickel, copper and zinc, fell by 40% during the global financial crisis in 2008, the price of gold increased by 6% (Shafiee and Topal, 2010). The demand and price of gold continues to rise as there is: (i) an escalation in the demand for gold jewellery, driven by private consumers from India and China, who view gold as a personal investment to safeguard against inflation; (ii) an ever increasing demand for electrical products, such as mobile phones and computers, which contain gold-based components; and (iii) a re-emergence of gold as a secure investment, replacing more volatile assets such as stocks, currencies and real estate (Shafiee and Topal, 2010).

The high demand for gold has raised the price to USD$ 1721 per troy ounce (1st of March 2012), increasing the need for new mineable gold deposits. In spite of the progress achieved using geochemical and geophysical techniques, exploration for new gold deposits is technically challenging, thus the discovery of new world-class deposits is extremely rare. One reason for this is that outcropping deposits and those with obvious geophysical and geochemical signatures have already been discovered. Thus, new field- and laboratory-based techniques are required that will complement existing technologies and provide additional information to narrow-down targeting for green- and brown-field exploration. Biosensor- and bioindicator techniques, based on microbially-mediated processes, may provide a solution that can lead to increased exploration success.

The dwindling number of newly discovered, close to surface, high-grade gold deposits has also escalated the mining industries interest in the processing of low-grade gold ore. Significantly lower grade deposits can be exploited than was financially feasible even ten years ago, assisted by the high gold price. Hence, mining and exploration companies are shifting their focus on finding low-grade deposits close to surface, rather than high-grade deposits deep below the surface. The emphasis on large, low-grade resources means that ever-increasing volumes of ore require processing. This has dramatically increased the demand for cyanide, in the United States approximately 1 million tons of cyanide is produced annually, of which the mining industry uses 56% (Cipollone et al., 2008). Although the cyanidation process is well accepted by the mining industry, because of its efficiency and low cost, there is concern that large-scale cyanide transport may potentially lead to environmental problems, an intensification of regulations and an increase in public opposition to the practice (Akcil and Mudder, 2003; Olson, 1994). In Australia, for example, approximately 60,000 tons of cyanide are transported annually on Australian roads for use in mines all over the country (Australian Government, Department of Health and Ageing, 2010). Moreover, many of the gold deposits discovered today are 'problematic' with respect to readily extractable gold, and economical gold recovery can often not be fully achieved by conventional processing technologies (Gasparrini, 1983; Harris, 1990; Williams, 1993). It is thus beneficial to the minerals industry to develop environmentally friendly, cost-efficient gold processing techniques and methods for the on-site production of gold-solubilising agents.

Metal cycling is greatly influenced by microorganisms via both direct and indirect mechanisms (*e.g.*, Ehrlich, 2002). Some metals are essential for the growth of microorganisms, such as magnesium, sodium, potassium, iron, cobalt, copper, molybdenum, nickel and zinc (*e.g.*, Ehrlich, 2002; Madigan and Martinko, 2006; Gadd, 2010). Others can be utilised by microorganisms as energy sources, such as oxidised or reduced species of iron, arsenic, manganese, vanadium, selenium and uranium (*e.g.*, Ehrlich, 2002). Some metals have no known function within cells but can be accumulated, for example caesium, aluminium, cadmium, mercury and lead (*e.g.*, Silver, 1996; Gadd, 2010). Rapid advancements in microscopic and molecular microbial techniques have facilitated an evolution in our comprehension of how metals, specifically gold, interact within the environment (*e.g.* Brugger et al., 2010; Reith et al., 2010; Ciobanu et al., 2011). This has resulted in a paradigm shift: gold is no longer thought of as a stable and inert substance under surface conditions (Reith et al., 2007). Microorganisms have been shown to play a greater role in the solubilisation, dispersion, re-concentration and the formation of gold mineralisation, than previously thought (Reith et al., 2007; Southam et al., 2009).

Fundamental knowledge of the interactions between gold and microorganisms in the environment, coupled with cutting edge technologies can now be used to develop new methods for gold exploration, the processing of gold ore and the remediation of mine waste (Fig. 1). For gold exploration the use of specific bioindicators and biosensors that will improve our ability to discover gold deposits should be investigated. The processing of gold ores involving the use of cyanide-producing microorganisms using *in situ* leaching methods should be further explored. Lastly cyanide-utilising microorganisms may be used for cyanide biodegradation. These opportunities highlight the importance of fundamental research and its implementation into industrial applications (Fig. 2).

2. Bioindicators for gold exploration

Geochemical exploration is commonly based on the analysis of trace amounts of the metals of interest (*e.g.* gold) and suitable pathfinder elements (*e.g.* arsenic, bismuth, molybdenum, selenium and silver) in rocks, soils, specific minerals or biological media. For example, trees such as the snappy gum tree (*Eucalyptus brevifolia*) can provide a convenient and accessible method of identifying valuable mineral deposits at depth (Dunn, 2007; Reid and Hill, 2010). Here, samples of vegetative material are collected, analysed for metals and the data is then used to determine the underlying mineralisation. In contrast, bioindicators rely on organisms that are resident in the mineralised zone and/or that are sensitive to metal. Hence, the presence of these organisms can indicate the occurrence of a specific metal contents derived from a buried ore body. The use of microorganisms as bioindicators for mineral exploration has remained an underexplored avenue, but the increasing sensitivity and decreasing cost of molecular methods make it possible to envisage the use of bioindicators as a new standard method in mineral exploration.

Microorganisms vastly outnumber and exhibit greater genetic diversity than macroorganisms, hence, the use of microorganisms as bioindicators holds many advantages. Modern molecular techniques have facilitated the generation of detailed phylogenetic profiles of environments that can be coupled with geochemical information to give a complete biological and geochemical picture of the system. Techniques such as terminal-restriction fragment length polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE), thermal gradient gel electrophoresis (TGGE), single strand conformation polymorphism (SSCP), and, more recently, microarrays and third generation DNA sequencing technologies, allow for the high-throughput analysis of microbial communities. Such techniques are being used to monitor pollutants in numerous environments (Sharma et al., 2011), and can be adapted to aid in the detection of metal contaminants present in low concentrations (Reith and Rogers, 2007). The major advantages of using bioindicators as mineral exploration tools lie in: (i) the possibility that a multitude of different metals will be detectable simultaneously; and (ii) the ability of microorganisms to respond to very low concentrations of metals. Technically there are two possible avenues for the application of microbial bioindicators; these are: (i) comparison of microbial community profiles, known as 'phylogenetic fingerprinting';

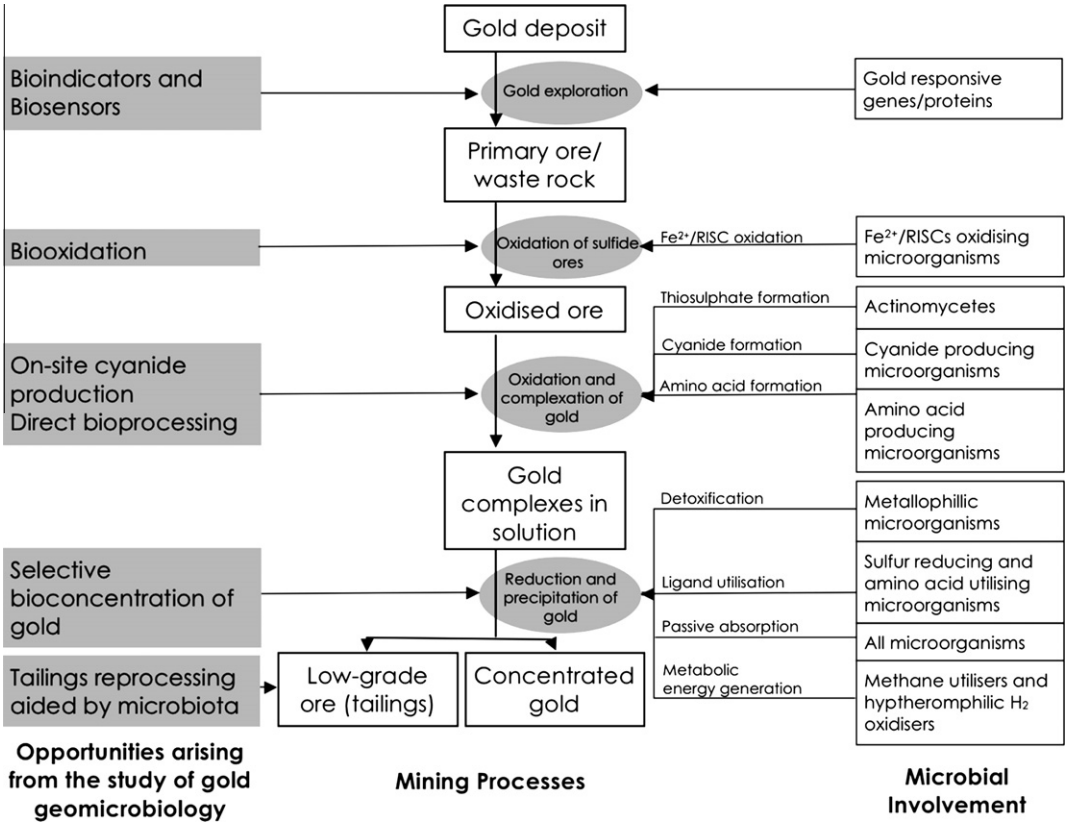

Fig. 1. Microbially driven opportunities in gold mining.

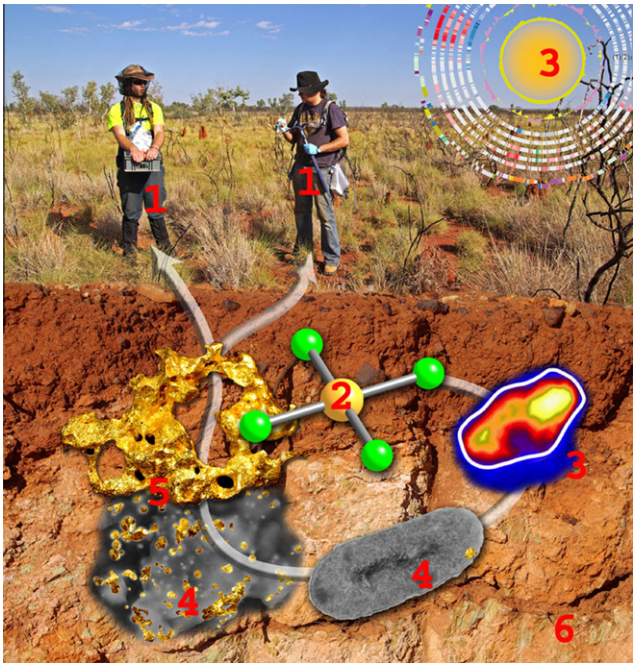

Fig. 2. Understanding geomicrobial gold cycling; *i.e.*, 2, solubilisation and transport; 3, bioaccumulation; 4, reductive biomineralisation; 5, secondary gold formation; helps mineral explorers (1) to find new gold deposits and provide new ore-processing approaches.

and, (ii) detection of specific genes, proteins and/or metabolites. Ultimately, the genetic profiling of an environment could be used to help determine the geochemical composition of a site.

2.1. Phylogenetic fingerprinting

Phylogenetic fingerprinting firstly involves identifying the microorganisms present in an environment. This can then be used to link the presence of these microorganisms or combinations of microorganisms to environmental parameters. The comparison of microbial community profiles to access environmental changes, such as the introduction of pollutants and heavy metals, is not a new idea and has been of interest to scientists for many years (reviewed in Hinojosa et al., 2010). Research into the phylogenetic fingerprinting of heavy metal containing soils is ongoing, and strong correlative link between the phylogenetic fingerprint of a given microbial community and the presence of metal contaminants has been established (Hinojosa et al., 2010).

The use of phylogenetic fingerprinting techniques for mineral exploration has been limited (Wakelin et al., 2012a). However, proof of concept has already been established in several recent studies. Using DGGE, Reith and Rogers (2008) demonstrated that the phylogenetic fingerprint of bacterial communities at the Tomakin Park gold mine (south-eastern New South Wales, Australia) was influenced by the presence of gold and its pathfinder elements. A similar correlation was also shown in another study on soils overlying a volcanogenic massive sulphide (VMS) deposit in the northeast of Western Australia. Here the investigation of the microbial community structure was coupled with soil geochemistry and biogeochemical analysis of plants (*i.e.*, *Acacia aeura*; Wakelin et al., 2012a). On the basis of DGGE data, soils overlying mineralisation displayed significant differences in the microbial community compared to soils outside of the mineralised zone (referred to as background soils; Wakelin et al., 2012a). This demonstrates that the microbial community profiles can be used to identify mineralised zones.

Recent developments in microarray analysis (PhyloChip and GeoChip) and DNA sequencing technologies have meant that microbial community profiles can be generated to cover a greater depth of microbial species than ever before (Rastogi and Sani, 2011). For example, the PhyloChip is able to simultaneously detect more than 8000 strains of microorganisms (manufactures claim), while high-throughput sequencing methods theoretically can detect every single microorganism present in a sample. Wakelin et al. (2012b) integrated several techniques to gain a better understanding of the composition and function of bacterial communities from a sulphidic mine tailing dump located 600 km northeast of Perth, Western Australia. Differences in bacterial communities were first assessed with DGGE and then members of the community were further identified using the PhyloChip. The results from this study showed that mobile phase elements, such as sulphur, zinc, chloride and aluminium, were the dominant drivers of bacterial community structures at this site. Using PhyloChip, a type of microarray, the authors were able to identify a greater level of species richness than had been reported at similar sites in the past (Wakelin et al., 2012b). These results demonstrate how modern molecular techniques enable the assessment of microbial community structures at great depth. The use of microarrays and DNA sequencing technologies enhances our ability to link the phylogenetic fingerprint of an environment with various environmental parameters. In terms of gold exploration, our improved ability to resolve microbial community structures will aid in the development of gold bioindicators. Extending the use of the aforementioned molecular techniques will aid in future investigations by providing in depth microbial profiles, leading to the generation of specific phylogenetic fingerprints that are indicative of certain metal species. Ultimately, this will assist the mining industry in its gold exploration endeavours.

2.2. Detection of specific genes, proteins, and/or metabolites

The detection of specific genes, proteins and/or metabolites is possibly the most promising basis for the development of bioindicators for mineral exploration (Hu et al., 2007). Like antibiotic resistant genes, metal resistance genes are often located on plasmids, which are mobile genetic elements that are transferable between compatible microorganisms (Mergeay et al., 2009). This means that two completely different microorganisms may have the same mechanisms of metal resistance. Phylogenetic fingerprinting relies on the assumption that the presence of microbial genera, species or strains can be specific to certain environmental condition. If an indicator microorganism or set of microorganisms is not present it is assumed that these environmental conditions are not present. This may lead to the inadvertent dismissal of a positive site when in reality another species may have gained the ability to occupy that ecological niche, *e.g.* through the acquisition of a plasmid. Hence, the identification of genes and subsequently their proteins and metabolites may allow for more accurate profiling of an exploration site.

There are a range of genes that are associated with the ability of microorganisms to resist and detoxify heavy metals, for example: *sil* genes for resistance to silver (Woods et al., 2009); the *czc*, *cad* and *cad* genes for cadmium resistance (Hu and Zhao, 2007); *chr*, *ncc* and *mer* genes for resistance to chromium, nickel and mercury, respectably (Abou-Shanab et al., 2007). Other genes have been shown to be differentially abundant in the presence of metals, such as the *gol* genes in the presence of gold (Checa et al., 2007; Reith et al., 2009). Transcriptomics is a method of detecting and quantifying RNA, and can be used to find genes that are more or less abundant in the presence of an environmental parameter. Transcriptomics could be used to assess the overall expression of the aforementioned genes in an environment, serving as an indicator as to what metals are present within the system. Proteins could be used in a similar manner, utilising proteomics and mass spectrometry techniques, protein ligand assays could be developed to allow for on-site detection of metals. Finally, metabolomic techniques, which look at the metabolites that result from biochemical reactions within organisms, could also be used to access the 'chemical fingerprint' of cellular reactions involving metals (Schneider and Riedel, 2011).

The development of a successful bioindicator will doubtlessly require the ability to detect specific genes, proteins and/or metabolites from a number of microbial species. Hence, there is a necessity for more research to be undertaken into the composition of microbial communities from gold in mineralised and background soils using state-of-the-art transcriptomic, proteomic and metabolomic techniques. This will allow for the identification of microbial species that are closely associated with the presence of gold and the subsequent development of a system to identify genes, proteins and/or metabolites from a range of microorganisms important for the detection of gold.

3. Biosensors in gold exploration

Biosensors are analytical devices that are based on biological components and are developed to detect specific compounds (Luong et al., 2008). Research into biosensors has been focused around: the monitoring of blood glucose levels; the detection and quantification of pathogens, food toxins, illicit drugs and heavy metals; and biosafety (Luong et al., 2008). The basis behind the majority of these biosensing devices is the binding of an analyte to a protein, usually an enzyme, which induces a measurable change (*e.g.*, electrochemical, optical, thermometric, piezoelectric, or magnetic), which can then be converted into a useful reading. Because proteins are highly selective and sensitive towards specific ligands, protein-based biosensor units are also highly selective and sensitive towards the specific analyte. For instance, the protein CueR, sensitivity towards copper(II)-ions lay in the zeptomolar (10^{-21}) range (Changela et al., 2003).

The driving force behind the continued enthusiasm for biosensor technologies is due to the many benefits of using these systems over traditional detection methods, such as wet chemical tests or mass spectrometry. Biosensors can be developed into inexpensive devices that are simple to operate, *e.g.*, the electrochemical blood glucose biosensor (Wang, 2007). Specifically, the use of biosensing technologies over traditional techniques for mineral exploration holds value in the speed, portability and high selectivity of these devices. The development of biosensors for gold exploration will mean that exploration teams will be able to obtain gold concentrations from an environmental sample immediately, rather than weeks later, which is the current turn around time. In addition, biosensing devices may also aid in mineral processing where real-time in-line analysis of specific mineral components of ores could be determined, enabling real-time fine-tuning of the process to improve recovery and costs.

Of particular interest for the development of a gold biosensor is the bacterium *Cupriavidus* (*Ralstonia*) *metallidurans*. Originally isolated from metallurgical sediments in Belgium (Mergeay et al., 1978, 1985; Van Houdt et al., 2009), this bacterium has since been isolated from many countries including the Congo, Germany and Australia, always in close association with metal-rich environments (Goris et al., 2001). Using DGGE and cloning, *C. metallidurans* was found to be the key organism detected in biofilms on gold grains from three Australian sites (*e.g.* Fig. 3; Reith et al., 2006; 2010). Furthermore, focused ion beam-scanning electron microscopy (FIB-SEM) was used to visualise a biofilm composed of *C. metallidurans* and covering gold grains (Reith et al., 2010). Transcriptome

microarray analysis identified a number of genes (*gol*, *cop*, *mer*, *ars* and *cus*) in *C. metallidurans* strain CH34 which were more abundant upon cellular exposure to soluble gold compounds (Reith et al., 2009). In the presence of gold(I/III)-complexes *C. metallidurans* induces a gold-specific genetic region (Rmet_4682–4687) which were more abundant with gold(III)-complexes, than with 16 other metal ions (Reith et al., 2009). The close association of *C. metallidurans* with gold and its genetic response to the presence of gold makes this microorganism a particularly interesting candidate for the development of a gold biosensor. Gold-regulated genes have also been detected in other bacteria such as the *gol* genes of *Samonella typhimurium* (Checa et al., 2007; Cerminati et al., 2011).

A major consideration for the implementation of a gold biosensor is the solubilisation of gold from an exploration or processing sample. Hence for any biosensor to work, a suitable method for the extraction of gold from the sampling medium, *e.g.*, the soil, has to be developed. Gold, unlike other metals, does not form free ions in aqueous solution at surface conditions, but occurs as aurous (+I) and auric (+III) complexes and metallic gold nano-particles (Reith et al., 2007; Usher et al., 2009). Hence, the speciation (*i.e.*, oxidation state) of gold and concentration determines the genetic response of microorganisms to gold. For example, the minimal inhibitory concentration (MIC) for gold(III)-chloride in *C. metallidurans* is 2 μM, while for gold(I)-cyanide it is >200 μM (Reith et al., 2009; Grosse, pers. comm.). A sound understanding of the chemistry of gold complexes and its geochemical behaviour are required to be able to implement biosensors in mining scenarios. When these challenges are overcome, gold exploration can be conducted with never before experienced speed and accuracy.

4. Microbial gold processing

In any given ore or tailings dump gold is present in different physical and mineralogical forms: (i) free-milling native gold or electrum; (ii) fine-grained particles of native gold or electrum (0.1 to ~5 μm in size) within sulphides, oxides or gangue; (iii) invisible (or refractory) gold, hosted within sulphides (principally pyrite and arsenopyrite), either in the lattice or as nano-particles (Palenik et al., 2004); (iv) other gold minerals, *e.g.* Au-(Ag)-tellurides; and (v) (rarely) within other 'exotic' gold-carrier minerals (Harris, 1990).

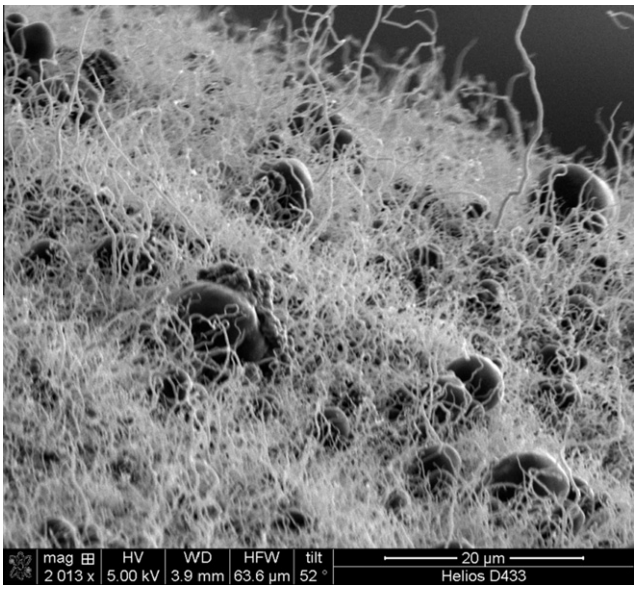

Fig. 3. Scanning electron micrograph of a microbial biofilm on the surface of a gold nugget.

Low gold recovery rates often result from gold being locked in minute inclusions or within the sulphide matrix – so-called 'invisible' or refractory gold (Cook and Chryssoulis, 1990). This gold cannot be recovered by conventional gravity or flotation methods. If a significant proportion of the total gold is 'invisible', then additional methods, such as roasting or biooxidation are used to treat the 'problematic' gold ores. An adequate understanding of the partitioning and mineralogical distribution of gold in an ore body is essential before designing a processing circuit, since the mineralogy of gold will directly impact on the technology chosen. Recent case studies are showing that concentrations of invisible gold vary with ore texture and that texturally-defined sub-populations of pyrite can be identified that have very different gold concentrations. Especially if biooxidation is to be used, the composition, grain size and texture of the matrix hosting the gold particles are of critical importance as these will influence accessibility pathways for the microorganism. An additional point to note here is that invisible gold is most commonly (though there are exceptions) hosted within arsenic-bearing pyrite or arsenopyrite, presenting a potential additional environmental risk if these minerals are to be roasted or bioleached.

Geological processes during ore formation, superimposed alteration and weathering will impact upon distribution, commonly leading to gold redistribution (*e.g.*, Sung et al., 2009). Mineral replacement processes leading to grain-scale redistribution and nucleation of nano-inclusions are increasingly recognised as critical in governing gold distributions in some ores (Tooth et al., 2011). Fluid-mineral reactions that couple dissolution with re-precipitation rates (CDRR; Putnis, 2009) are particularly productive in concentrating gold because they are driven by high transient porosity, in turn providing sites for precipitation of gold-minerals (Cook et al., 2009; Xia et al., 2009; Qian et al., 2010, 2011). Biological, and in particular microbial, activity is another factor that controls the nature and kinetics of gold redistribution in the near-surface to surface environment (Reith et al., 2007; Southam et al., 2009). Therefore, a solid understanding of the processes that control both the (bio)-geochemistry and mineralogy of gold forms the foundation for the development of innovations to improve existing processing technologies.

One of the key developments with respect to understanding the mineralogical distribution of gold is being able to bridge different scales of observation – *i.e.*, from whole deposit to a hand specimen (a few cm) and further down to the micro- and nano-scales in gold containing minerals. One powerful tool that can revolutionise the way in which ore minerals can be addressed at the nanoscale is FIB-SEM. FIB-SEM platforms allow high-resolution imaging, 3D-tomography and *in situ* preparation of thinned foils that can be examined using high resolution-transmission electron microscopy (HR-TEM). The application of FIB-SEM techniques to ore minerals is in its infancy (Ciobanu et al., 2011), but is likely to revolutionise our understanding of ore-forming and -modifying processes at the scale at which they occur. The type of sub-micron- to nanoscale information now obtainable, coupled with determination of trace element concentrations at sub-ppm levels by *e.g.* laser-ablation inductively-coupled mass spectroscopy (LA-ICP-MS), within host minerals (Fig. 4), provides critical data on the mineralogical distribution of gold, a pre-requisite for innovative application of any microorganisms in mineral processing.

The use of microorganisms in gold processing is an established technique (Brierley, 2008). Specifically, the pre-processing of gold-containing sulphide ores, in stirred tank biooxidation reactors has been applied to a variety of ores worldwide making it a commercial success (Rawlings et al., 2003). The biooxidation process enables the liberation of gold encapsulated in sulphide minerals, making the gold available for processing via traditional inorganic routes (Olson et al., 2003; Brierley, 2010). The extraction and re-concen-

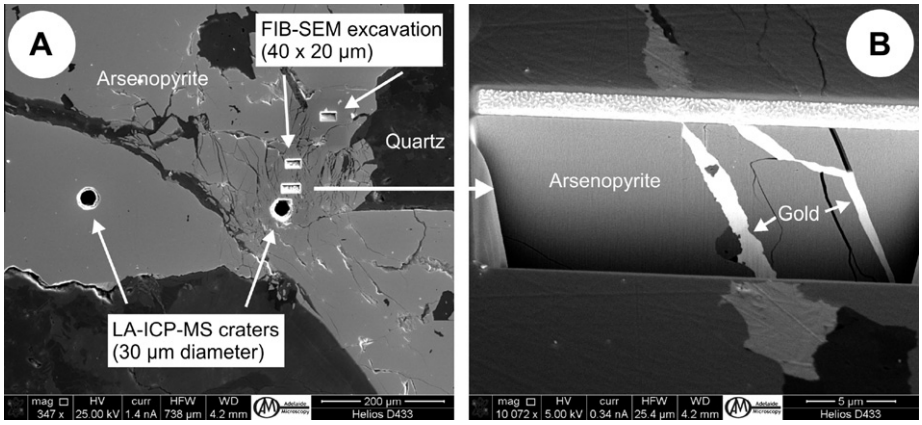

Fig. 4. FIB-SEM images. (A) LA-ICP-MS crates providing trace element compositional data and FIB-SEM excavation sites within a deformed grain of Au-bearing arsenopyrite. (B) Detail of FIB-SEM excavation across micron-scale remobilised gold grains filling brittle fractures in arsenopyrite.

tration of gold still requires cyanide leaching techniques and electrochemistry.

The biologically mediated mobility of gold is complex and diverse (Reith et al., 2007). Ranging from influences by iron and/or reduced inorganic sulphur compound (RISC) oxidising microorganisms, such as *Acidithiobacillus ferrooxidans* (Lengke and Southam, 2005), to the production of cyanide on gold-containing minerals by bacteria, such as *Chromobacterium violaceum* and *Pseudomonas aeruginosa* (Blumer and Haas, 2000; Fairbrother et al., 2009). Recent experiments have shown that the influence of microorganisms in gold solubilisation can be used to further develop the field of microbially mediated gold extraction. Four areas of potential applications of biotechnologies to gold processing and remediation are of particular interest: (i) the on-site production of cyanide by microbiota; (ii) the microbially mediated decomposition of cyanide; (iii) the use of microorganisms for gold bioleaching in heaps and stirred tank reactors; and (iv) the use of microorganisms for the *in situ* leaching of gold ores.

4.1. The microbially mediated production of cyanide

The processing of gold has traditionally relied on cyanide leaching and/or roasting, however, the use of these methods is becoming more technically and legislatively problematic for the mining industry. One option is to produce cyanide on site in bioreactors, and then use this cyanide in traditional processing methods. Cyanide is produced by microorganisms at the end of their growth phase as a by-product of the oxidative decarboxylation of glycine (Cipollone et al., 2008). Although cyanide production by microorganisms is a well-studied area it is unlikely that microorganisms may be able to provide the mining industry with an economic alternative to the chemical generation of cyanide (Campbell et al., 2001). The major problem with the microbial generation of cyanide is the rate of production and energy cost. However, the targeted production of cyanide directly onto the surface '*in situ*' of gold-containing minerals may help to overcome this problem.

4.2. The use of microorganisms for the in situ leaching of gold ores

An exciting opportunity, revealed by the observation of gold geobiology, is to use gold-targeting microorganisms to generate cyanide directly on the surface of the gold-containing minerals, limiting the necessity for transport and reducing the total quantity of cyanide required. Several studies have shown that microorganisms directly attach to the surface of gold, form biofilms and produce cyanide, which in-turn solubilises gold. For example, *C. violaceum* has been shown to rapidly form biofilms on the surfaces of gold-coated slides (Campbell et al., 2001). The production of cyanide by these attached microorganisms was then demonstrated to directly aid in the leaching of gold from both gold-coated slides and a gold concentrate that had prior treatment with biooxidation. This indicates that cyanide-producing microorganisms can be coupled with existing technologies, such as biooxidation, to assist in the leaching of gold or may also have potential for *in situ* bioleaching.

In a separate study, microcosm experiments were conducted using auriferous soils and vein-quartz material collected from the Tomakin Park gold mine (Reith and McPhail, 2006). Microcosms were either sterilised (abiotic controls) or not sterilised to allow the native microorganisms to grow during the experiment. Under oxic conditions gold was solubilised more rapidly in biologically active samples than abiotic controls. The results of this experiment demonstrated that microorganisms play a vital role in the solubilisation of gold and that microorganisms native to soils maybe useful for the *in situ* leaching of gold.

Gold uptake by microorganisms was investigated by Reith et al. (2009) mapping gold uptake throughout a microbial cell using synchrotron µ-X-ray fluorescence (µ-XRF). Coupled with results from transmission electron microscopy (TEM), the data showed that ionic gold from the cytoplasm is precipitated as nano-particulate, metallic gold in the periplasm. This study is unique, in that it serves as an indication that active reductive precipitation by bacteria may be used for the selective extraction of gold *in situ* via biosolubilisation and -concentration.

These studies indicate that the use of microorganisms for gold processing has extensive potential. *In situ* leaching has many economical and environmental advantages over open-cut and underground mining. As the minable deposits of gold ore close to the Earths surface diminish the requirement for methods to feasibly extract gold from greater depths will become a priority for the mining industry. From the already established knowledge on cyanide-producing microorganisms and their ability to solubilise gold there is no doubt that *in situ* leaching of gold will be a very promising up-and-coming technology.

4.3. The microbially mediated destruction of cyanide

Currently, the mining industry has to remediate large quantities of waste cyanide. Many plants, algae, fungi and bacteria possess the ability to break down cyanide into less toxic compounds and may present a method of cyanide remediation for the mining industry (reviewed by Cipollone et al., 2008). Cyanide is removed

from industrial waste by chemical, physical or biological methods, such as UV exposure, oxidation or alkalisation (Dzombak et al., 1996; Mudder and Botz, 2004). In mining, cyanide wastes are transferred to tailings ponds where they are remediated. However, these strategies are only effective for cyanide that is either free or only weakly associated with metals and are often associated with high running costs (Gupta et al., 2010). A viable alternative for chemically based technologies is the biological, mainly microbiological, mediated breakdown of cyanide (Desai and Ramakrishna, 1998).

There are a number of reports on the bioremediation of cyanide wastes from mine sites (Akcil and Mudder, 2003; Álvare et al., 2004; Ebbs, 2004; Dash, 2009). The major issue with implementing cyanide bioremediation has been stated as the costs associated with setting up the operation (Álvare et al., 2004; Ebbs, 2004). Akcil and Mudder (2003) eloquently stated "A primary disadvantage of biological treatment is the lengthy and sometimes costly laboratory and pilot plant scale programs required to develop and design a full-scale treatment system for each different application".

To make the bioremoval of cyanides economically viable it is essential to make the process as efficient as possible. The biological degradation of cyanide can occur via four general pathways: oxidative; reductive; hydrolytic; and substitution/transfer (Cipollone et al., 2008). Each of these pathways is sensitive to external parameters such as aeration, cyanide concentration and pH (Ebbs, 2004). Hence, understanding the geochemistry of the gold-containing minerals is essential for designing economically feasible biological systems for the degradation of cyanide (Dash et al., 2009). Once we have developed a greater understanding of cyanide wastes bioremediation plants can be implemented with greater ease. The trail and error system for optimising bioremediation plants today will be replaced with a sound scientific understanding of the processes taking place. This will lead to a decrease in set-up costs and greater uptake of the technology.

4.4. The use of microorganisms for gold bioleaching in heaps and biooxidation stirred tank reactors

Bioleaching of gold-containing ores in heaps and biooxidation stirred tank reactors is a well-established technique with potential for further optimisation as molecular techniques advance. For many years the consensus amongst researchers was that *A. ferrooxidans* was the most important mesophilic bioleaching microorganism (Lundgren and Silver, 1980; Brierley, 1982). However, with the development culture independent molecular techniques for the identification and quantification of microorganisms it was found that *A. ferrooxidans* was less important than was initially thought (Rawlings et al., 1999). As researchers shifted their understanding of microbial communities in bioleaching systems, mesophilic bacteria became the subject of much research. It is now being shown that moderately thermophilic microorganisms, namely archaea, are of great importance to the development of efficient bioleaching technologies (Franzmann et al., 2005; Plumb et al., 2008).

The investigation of the microorganisms involved in bioleaching is a promising area of research. Each ore hosts a unique population of microorganisms. When these ores are subjected to bioleaching this native population of microorganisms may be used to extract the metal of interest, a method which is often employed in heap leaching. A known mixture of microorganisms can also be used to inoculate the ore, such as used in the BIOX® processes. Monitoring these population during the leaching processes and assessing their effects of metal leaching on such a large scale has proven to be a complex endeavour. Hence, scientists have not resolved what affect our attempts to manipulate naturally occurring populations have on processing efficiency.

With the development of high-throughput molecular techniques, the routine undertaking of such complex studies is becoming a reality and will undoubtable aid in the advancement of bioleaching technologies. The Escondida mine in Chile is the world's largest producing copper mine, and processes its run-of-mine (ROM) sulphide ore (0.3–0.7% Cu) by heap bioleaching (Demergasso et al., 2003). A recent study investigated how the microbial population responded to changes in environmental factors over ~650 days (Demergasso et al., 2003). A relatively basic technique (real-time PCR) was used to quantify the microorganisms, which meant that only a few species or strains could be investigated. Nonetheless, several relevant findings were discovered. For example, the solvent extraction process impacted on the ability to inoculate the raffinate solution. This is an example of a finding that can now be investigated further and will eventually lead to more effective bioleaching. As molecular techniques evolve more of these types of studies can be carried out with greater detail and depth leading to the optimisation of commercial bioleaching.

5. Conclusions and outlook

With the ever-expanding capabilities of molecular analysis techniques our understanding of biological systems will become more complete, directly affecting our ability to adapt and apply this knowledge to industrial systems. Using state-of-the-art technologies a preliminary model of the movement of gold in the environment has been elucidated. With the aim of improving exploration and processing of gold, an understanding of the distribution of gold and the microbial processes involved in controlling gold deposition and mobilisation is of importance. This is achievable because of the resolving power of today's generation of micro-analytical instrumentation. To achieve these insights and develop the gold mining technologies a multifaceted, multidisciplinary approach is required. State-of-the-art technology will advance our fundamental understanding of microbial populations and the processes involved in the bio-solubilisation and re-precipitation of gold in ores, natural waste rock piles as well as determine the conditions for the optimal recovery of gold via biological mechanisms. Microarray-, proteomic- and culture-based techniques in combination with electron microscopic and synchrotron spectroscopic techniques will lay the foundations for novel biotechnologies for gold exploration and processing.

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