Biorecovery of gold

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Recovery of ionic and metallic gold (Au) from a wide variety of solutions by selected species of bacteria, yeasts, fungi, algae, and higher plants is documented. Gold accumulations were up to 7.0 g/kg dry weight (DW) in various species of bacteria, 25.0 g/kg DW in freshwater algae, 84.0 g/kg DW in peat, and 100.0 g/kg DW in dried fungus mixed with keratin material. Mechanisms of accumulation include oxidation, dissolution, reduction, leaching, and sorption. Uptake patterns are significantly modified by the physicochemical milieu. Crab exoskeletons accumulate up to 4.9 g Au/kg OW; however, gold accumulations in various tissues of living teleosts, decapod crustaceans, and bivalve molluscs are negligible.

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Extraction of gold from solutions is under active investigation using a variety of physical, chemical, and biological processes. Recovery of ionic gold from dilute solutions usually involves either precipitation by zinc dust, carbon adsorption, solvent extraction, or ion exchange resins. All of these are of low selectivity and comparatively expensive. Chemical methods for the recovery of gold from ores include cyanidation and thiourea leaching, which present environmental and health risks. Biorecovery of dissolved gold from solution presents fewer environmental risks than chemical methods, and is documented for microorganisms, algae, water ferns, peat, alfalfa, seaweeds, fungi, yeasts, and crab exoskeletons. This account briefly reviews the potential of living and dead plants and animals to accumulate gold from solution, and some of the processes involved—including biooxidation, dissolution, bioreduction, bacterial leaching, and biosorption.

Gold uptake by microorganisms, fungi, and higher plants

Biomining processes are used successfully on a commercial scale for the recovery of gold and other metals, and are based on the activity of obligate chemoautotrophic bacteria that use iron or sulfur as their energy source and grow in highly acidic media. Biooxidation of difficult to treat gold-bearing arsenopyrite ores occurs in aerated, stirred tanks and rapidly-growing, arsenic-resistant bacterial strains of Thiobacillus ferrooxidans (Beijerinck), Leptospirillum ferrooxidans (Beijerinck), and Thiobacillus ferrooxidans (Beijerinck). These bacterial species obtain their energy through the oxidation of ferrous to ferric iron (T. ferrooxidans, L. ferrooxidans) or through the reduction of inorganic sulfur compounds to sulfate (Thiobacillus spp.). Monetary costs of biooxidation are reported to be about 50% lower than roasting or pressure oxidation. Adding Thiobacillus ferrooxidans into the thiourea leaching solution produces a 20% increase in the extraction of gold. The reaction describing gold dissolution in an acidic solution of thiourea in the presence of ferric ion is described by Kai et al. as:

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\text{Au}^+ + \text{Fe}^{3+} + 2\text{CS(NH}_2\text{)}_2 \rightarrow \text{Au[CS(NH}_2\text{)}_2]^2+ + \text{Fe}^{2+}
\]

The use of bacteria in pretreatment processes to degrade recalcitrant gold-bearing arsenopyrite ores and concentrates is well established. Recalcitrant ores are those in which the gold is enclosed in a matrix of pyrite and arsenopyrite, and cannot be solubilized by direct cyanidation. Bacterial decomposition of arsenopyrite assists in opening the molecular mineral structure, permitting access of the gold to cyanide. However, greater quantities of cyanide are required to solubilize gold after bacterial treatment when ores contain high quantities of gold. A possible cause of this excessive cyanide is the presence of the enzyme rhodanese, produced by Thiobacillus caldus (Beijerinck), a common species of bacterium encountered in biooxidation facilities. Optimum microbiological leaching by Thiobacillus spp. and Sulfolobus spp. of refractory sulfide ores for

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recovery of gold in tanks is possible under controlled conditions of pH, dissolved oxygen, carbon dioxide, sulfur balance, redox potential, toxic metal concentrations, and rate of leaching.

Several species of Fe$^{3+}$-reducing bacteria (Bacteria spp., Archaea spp.) can precipitate gold by reducing Au$^{3+}$ to Au$^+$ with hydrogen as the electron donor. Rate of bacterial oxidation by Thiobacillus ferroxidans and Leptospirillum ferroxidans of three South African refractory gold ores of varying gold-arsenopyrite composition was dependent mainly on crystal structure. These gold ores were classified as refractory due to the presence of gold inclusions in arsenopyrite and pyrite, and submicroscopic gold in arsenopyrite. Refractory gold occurs at sites which are preferentially leached by the bacteria. The rate of gold liberation from sulfides is enhanced during the early stages of bacterial oxidation. Defects in crystal structure influence the rate of biooxidation and are directly related to the crystal structure of the sulfide mineral, the crystallographic orientation of the exposed surfaces, and differences in chemical composition and mechanical deviations in the crystals. Pretreatment of refractory gold concentrates with the bacteria Thiobacillus ferroxidans ultimately results in sulphur and sulphide oxidation by ferric ions from bacterial oxidation of ferrous ions. The maximum concentration of attached Thiobacillus increases with increasing concentration of Fe$^{2+}$ and decreases with increasing size of the refractory gold concentrate particles. In Chile, which produced 30,000 kg of gold in 1990, Thiobacillus ferroxidans was used to recover gold from a complex ore under laboratory conditions. The ore contained 8.2% Fe, 0.78% Cu, 0.88% As, and 3.5 g Au/ton, with pyrite, hematite, arsenopyrite, and chalcopyrite as the main metal-bearing minerals. Initial gold recovery by conventional cyanidation on a crushed ore sample was 54%; concentration by flotation improved recovery to 56%. Concentrated samples (17.0 g Au/ton) were leached in reactors at pH 1.8. In the presence of bacteria, all dissolved iron was present as ferrie iron; gold recovery by cyanidation increased from 13% for the initial concentrate to 97% after 10 days of bacterial leaching. To further increase gold recovery, flotation tailings were submitted to cyanidation.

Some microorganisms isolated from gold-bearing deposits are capable of dissolving gold; dissolution was aided by the presence of aspartic acid, histidine, serine, alanine, glycine, and metal oxidants. Bacteriform gold is well known, with uptake of Au$^{3+}$ from chloride solutions documented for at least seven genera of freshwater cyanobacteria. Some bacteriform gold is biogenic—the result of precipitation by bacteria—and may be useful indicators of gold deposits and of processes of gold accumulation. Plectonema terebrans Bornet, a species of filamentous marine cyanobacteria, accumulates gold in its sheath from an aqueous solution of AuCl$_4^-$.

Sheaths are among the few structures likely to be preserved in some form in microfossils of ancient bacteria. In marine media, it is expected that AuCl$_4^-$ (2.0 g Au/l) will form AuCl$_4^-$, Au$^{2+}$, and AuCl$_2$. Biosorption of Au$^{3+}$, as AuCl$_4^-$, by dried Pseudomonas strains of bacteria was inhibited by palladium, as Pd$^{2+}$, and possibly other metal ions.

Gold adsorption from cyanide solutions by dead biomass of bacteria (Bacillus subtilis), fungus (Penicillium chrysogenum Thom), or seaweed (Sargassum fluitans Linnaeus) at pH 2.0 were 1.5 g Au/kg DW for bacteria, 1.4 g/kg DW for fungus, and 0.6 g Au/kg DW for seaweed. Anionic AuCN$^{-}$ adsorption was the major mechanism in gold biosorption from cyanide solutions, being most efficient at lower pH values. L-cysteine increased gold-cyanide biosorption of Bacillus, Penicillium, and Sargassum. At pH 2.0, the maximum gold uptakes were 4.0 g Au/kg DW for bacteria, 2.8 g/kg DW for fungus, and 0.9 g/kg for seaweed, or 150-250% greater than in the absence of cysteine. The anionic gold cyanide species were adsorbed ionize functional groups on cysteine-loaded biomass; deposited gold could be eluted from gold-loaded biomass at pH 5.0.

Gold-resistant strains of bacteria that also accumulate gold are documented, although the fundamental mechanism of resistance to gold in microorganisms is not known or understood. One strain of Burkholderia (Pseudomonas) cepacia Burkholder contained millimolar concentrations of Au$^+$ thiolates. Burkholderia cells were large, accumulated polyhydroxybutyrate and gold, and excreted thiorin, a low molecular weight protein into the culture medium. This effect was not observed with the Au$^+$ complexes tested, which were reduced to metallic gold in the medium. Gold-resistant strains of fungi and heterotrophic bacteria are also known.

Rapid recovery of gold from gold-thiourea solutions was documented for waste biomass of yeasts (Saccharomyces cerevisiae), cyanobacteria (Spirulina platensis [Nordst]), and bacteria (Streptomyces erythralus [Waksman]). The process is pH-
dependent for yeast and bacteria, and pH-independent for Spirulina. Of all strains of microorganisms examined, Spirulina platensis has the highest affinity and capacity for gold, even at low pH values. Gold uptake by Spirulina was 7.0 g Au/kg biomass DW in 1-2 hr at pH 2.0, and about 3.0 g Au/kg DW in 15 min at pH 2 through \(^{14}\).

Metabolically active fungal cells of Aspergillus fumigatus Fresen and A. niger Tiegh removed gold from cyanide leach liquor of a Brazilian gold extraction plant more efficiently than did dried fungal biomass or other species of Aspergillus tested. These two species of fungi removed 35 to 37% of gold from solutions containing 2.8 mg Au/I in 84 hr \(^{26}\). Gold removal from cyanide-containing solutions is documented for a strain of Aspergillus niger, a fungus isolated from the gold extraction plant at Nova Linda, Brazil \(^{26-28}\). The leach liquor contained, in mg/l, 181.0 cyanide, 1.3 gold, 0.4 silver, 7.1 copper, 5.2 iron, and 4.5 zinc. After 60-72 hr of incubation, A. niger removed from solution, probably by adsorption, 64% of the gold, 100% of the silver, 59% of the copper, 80% of the iron, and 74% of the zinc; all gold was removed after 120 hr. Use of this fungus to develop a bioprocess to reduce metal and cyanide levels as well as recovery of valuable metals shows promise \(^{29-28}\). Uptake patterns of gold from Au\(^{3+}\) solutions by dead fungal biomass followed mathematical uptake models of Langmuir and Freundlich; biomass was prepared from the fruiting body of a mushroom collected from the forests of Kerala, India \(^{29}\). Dried fungus, Cladosporium cladosporoides Fresen, mixed with keratinous material of natural origin to form a bead, proved effective in absorbing gold from solution \(^{12}\). The biosorptive beads adsorbed 100.0 g Au/kg beads from a solution containing 100.0 mg Au/I. Maximum biosorption of 80% occurred at acid pH (1-5) in less than 20 min. The biosorptive beads degraded in soil in about 140 days. The beads also removed 55% of the gold from electrolating solutions containing 46.0 mg Au/I, with observed gold loading capacity of 36.0 g/kg beads \(^{12}\). Dried biosorptive encapsulated in polysulfone were prepared from microorganisms isolated from pristine or acid mine drainage environments \(^{11}\). Biosorptive material rich in exopolysaccharides from the acid mine drainage site bound Au\(^{3+}\) three times more effectively than did other materials, and removed 100% of the Au\(^{3+}\) from solutions containing 1.0 mg Au/I within 16 hr at 23°C and pH 3.0.

Algal cells, alive or dead, rapidly accumulate Au\(^{3+}\) and begin to reduce it to Au\(^{+}\) and Au\(^+\) within 2 days \(^{33}\). Uptake of Au\(^{3+}\) by Chlorella vulgaris Beijerinck, a unicellular green alga, from solutions containing 10.0 or 20.0 mg Au\(^{3+}\)/I is documented \(^{31}\). Chlorella accumulated up to 16.5 g Au/kg DW. Inactivating the algal cells by various treatments resulted in some enhancement in uptake capacity over the pristine cells. Inactivation by heat treatment yielded up to 18.8 g/kg DW; for alkali treatment, this was 20.2 g/kg DW; for formaldehyde treatment, 25.5 g/kg DW; and for acid treatment, 25.4 g/kg DW. Elemental gold (Au\(^+\)) was measured by X-ray photoelectron spectroscopy on the cell surface, indicating that a reduction had occurred \(^{21}\). Studies with living Chlorella vulgaris suggest that accumulated Au\(^{3+}\) is rapidly reduced to Au\(^{+}\), followed by a slow reduction to Au\(^+\). With dead algae, Au\(^+\) initiates a seeding process which results in the formation of elemental gold.

Sequestering metal ions using living or dead plants is a proposed economical means of removing gold and other metals via intracellular accumulation or surface adsorption. However, in the case of live plants, this is frequently a relatively slow and time-consuming process. Nonliving plant material for surface adsorption offers several advantages over live plants, including reduced cost, greater availability, easier regeneration, and higher metal specificity. In South African mining effluents, gold usually ranges between 1 and 10 mg/I. In studies of 180-min duration, dried red water ferns, Azolla filiculoides Lamark, removed 86 to 100% of Au\(^{3+}\) from initial solution of 2 to 10 mg Au\(^{3+}\)/I, increasing with increased initial concentration of Au\(^{3+}\) (ref. 22). The biomass gave >95% removal efficiency at all biomass concentrations measured. Optimum (99.9%) removal of gold occurred within 20 min at pH 2, 42% removal at pH 3 and 4, 63% at pH 5, and 73% removal at pH 6; removal efficiency seemed independent of temperature \(^{22}\). Similar results were observed with four species of ground dried seaweeds (Sargassum sp., Gracilaria sp., Eisenia sp., and Ulva sp.) \(^{25}\). Treated seaweeds removed 75-90% of the gold within 60 min at pH 2 from solutions containing 5.0 mg Au\(^{3+}\)/I. Gold (Au\(^{3+}\)) can be sequestered from acid solutions by dead biomass of a brown alga, Sargassum natans (Linnaeus), and deposited in its elemental form, Au\(^{2+}\) \(^{24}\). The cell wall of Sargassum was the major locale for gold deposition, with carbonyl groups (C=O) playing a major role in binding, and N-containing groups a lesser role. Like activated carbon, the biomass of Sargassum natans is extremely porous, reportedly more than most biomaterials, and accounts, in part,
for its ability to accumulate gold\textsuperscript{24}. Dried ground shoots of alfalfa, Medicago sativa Linnaeus, were effective in removing gold from solution\textsuperscript{3}. The accumulation process involved the reduction of Au\textsuperscript{3+} to colloidal Au\textsuperscript{0}, and was most efficient at elevated temperatures and acidic pH. In solutions containing 60 mg Au\textsuperscript{3+}/l, about 90\% of the Au\textsuperscript{3+} was bound to dried alfalfa shoots in about 2 hr at pH 2 and 55\^\circ C. The mechanism to account for this phenomenon is unknown, but may involve reduction of Au\textsuperscript{3+} to Au\textsuperscript{0}, the latter being unstable in water to form Au\textsuperscript{0} and Au\textsuperscript{3+} (ref. 4). Dried peat from a Brazilian bog accumulated up to 84.0 g Au/kg DW within 60 min from solutions containing 30.0 mg Au\textsuperscript{3+}/l (ref. 23).

**Gold uptake by aquatic macrofauna**

Except for crab exoskeletons, gold recovery from the medium by various species of living mollusces, crustaceans, and fishes is negligible.

Certain chitinous materials, such as exoskeletons of the swamp ghost crab, Ucides cordatus (Linnaeus), can remove and concentrate gold from anionic gold cyanide solutions over a wide range of pH values\textsuperscript{35}. The maximum AuCN\textsuperscript{-} uptake occurred at pH 3.7, corresponding to a final value of 4.9 g Au/kg DW; exoskeletons burnt in a non-oxidizing atmosphere removed 90\% of the gold at pH 10, phenolic groups created during the heat treatment seemed to be the main functional group responsible for AuCN\textsuperscript{-}; binding by burnt acid-washed crab shells\textsuperscript{36}.

Bioconcentration factors (BCFs) were recorded for carrier-free \textsuperscript{198}Au (physical half-life of 2.7 days) in freshwater organisms after immersion for 21 days in a medium containing 25,000 pCi/l = 675,700 Bq/l\textsuperscript{35}. In goldfish, Carassius auratus (Linnaeus), the highest BCFs measured were <1 in muscle (i.e., less than 675,700 Bq/kg FW muscle), 10 in viscera, and 9 in whole fish. In the freshwater winged floaters clam, Anodonta nataliiana Lea, the maximum BCF was 7 in soft parts; for crayfish (Astacus sp.), BCFs were <1 in muscle and 14 in viscera. For marine organisms immersed for 26 days in synthetic seawater containing 33,000 pCi/l = 891,900 Bq/l, maximum BCFs measured were 4 in muscle and 16 in viscera of the red crab, Cancer productus Randall, 11 in soft parts of the butter clam, Saxidomus giganteus (Deshayes), 12 in soft parts of the common mussel, Mytilus edulis Linnaeus, and <1 in muscle and 1 in a whole gobid fish, the longjaw mudsucker, Gillichthys mirabilis Cooper\textsuperscript{27}. Maximum stable gold concentrations recorded in soft tissues of marine mollusces and crustaceans ranged from 0.3 to 38.0 \mu g Au/kg DW; for fish muscle, the mean concentrations were 0.1 \mu g/kg DW and 2.6 \mu g/kg ash weight\textsuperscript{37}. In studies with the eastern oyster, Crassostrea virginica (Gmelin), the blue crab, Callinectes sapidus Rathbun, and the mummichog Fundulus heteroclitus (Linnaeus), an estuarine cyprinodontiform fish, all species were exposed in cages under field conditions to sediment-sorbed, carrier-free, \textsuperscript{198}Au\textsuperscript{3+}. The maximum level of radiogold in the caged organisms was detected in oysters 17 hr after contact with \textsuperscript{198}Au-spiked sediments. Indigenous organisms collected 41 hr after contact with the \textsuperscript{198}Au-labeled sediments contained no detectable radioactivity\textsuperscript{38}. In a 25-day study with blue crab, northern quahog clam Mercenaria mercenaria (Linnaeus), and the sheepshad minnow Cyprinodon variegatus Lacepede, all species were maintained in a 1000-l aquarium containing bentonite clay and seawater spiked with carrier-free \textsuperscript{199}Au (physical half-life of 3.2 days), as AuCl\textsubscript{3}: crabs accumulated the most radioactivity, followed by clams, clay, and fish, in that order\textsuperscript{39}.

Bioconcentration factors (BCFs) for metals and aquatic organisms derived from carrier-free radio-tracers in the medium are probably artificially high, and should be interpreted with caution\textsuperscript{35,36}. For metals it is a general observation that high BCFs are associated with low concentrations in the medium, and that BCFs are especially high when they are derived from carrier-free radioisotopes. Typically, BCFs for metals and other chemicals studied reach a plateau before declining with increasing concentrations in solution\textsuperscript{35,36}. The maximum concentration of stable gold measured in tissues of living marine organisms was 38.0 \mu g/kg FW\textsuperscript{35}.

**Conclusion**

Gold recovery by selected species of bacteria, algae, fungi, yeasts, and higher plants from dilute solutions under controlled physicochemical conditions seems economically viable, with concentrations up to 100 g Au/kg DW documented. Dried crab exoskeletons also show promise for commercial gold recovery from solution (4.9 g Au/kg DW); however, gold uptake by living species of aquatic macrofauna seems negligible. The mechanisms of accumulation which include oxidation, reduction, dissolution, leaching and sorption are not known with certainty and merit additional research effort.

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References
