Review

Microbe-mediated sustainable bio-recovery of gold from low-grade precious solid waste: A microbiological overview

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ABSTRACT

In an era of electronics, recovering the precious metal such as gold from ever increasing piles of electronic-wastes and metal-ion infested soil has become one of the prime concerns for researchers worldwide. Biological mining is an attractive, economical and non-hazardous to recover gold from the low-grade auriferous ore containing waste or soil. This review represents the recent major biological gold retrieval methods used to bio-mine gold. The biomining methods discussed in this review include, bioleaching, bio-oxidation, bio-precipitation, bio-flotation, bio-flocculation, bio-sorption, bio-reduction, bio-electrometallurgical technologies and bioaccumulation. The mechanism of gold bio-recovery by microbes is explained in detail to explore its intracellular mechanistic, which help it withstand high concentrations of gold without causing any fatal consequences. Major challenges and future opportunities associated with each method and how they will dictate the fate of gold bio-metallurgy from metal wastes or metal infested soil bioremediation in the coming future are also discussed. With the help of concurrent advancements in high-throughput technologies, the gold bio-exploratory methods will speed up our ways to ensure maximum gold retrieval out of such low-grade ores containing sources, while keeping the gold mining clean and more sustainable.

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Introduction

Over the years, the biological aspects of gold (Au) occurrence and extraction from low grade gold ore has always fascinated the researchers worldwide. The gold is mined globally at a large scale with China being the leading gold producing nation followed by Australia and Russia (Fig. 1, Ober, 2018). Recent study has modelled the full dynamics of global statics to reveal the adverse implications of vigorous gold mining and pointed at the possibility of total consumption of worldwide high grade gold mines stocks by 2030 (Sverdrup et al., 2012). So, researchers are finding solution to exploit available low-grade ores which lie unexplored inform of undervalued gold mines or resides in dumped electronic wastes (e-waste), waiting to be tapped on its valuable potential. Evidently, various parts of electronic devices require the use of various precious metals like gold due to its property of being inert and non-corrosive. In an era of electronics, outdated electronic devices easily get replaced by the advanced/or updated version, but leaving the former to be discarded (along with valuable metals enclosed within), which has catapulted the alarming issue of ever-increasing e-waste. Retrieving the precious metals from the discarded e-waste, thus become very important in terms of reclaiming the used precious metals back, which however is a quite challenging task due to lack of suitable sustainable technology. Conventionally, e-waste-based gold retrieval is done using the chemical methods, which however, is ill-famed for its hazardous nature, so non-toxic sustainable alternatives like microbe based biological methods would prove to be a promising alternative in long run.

In an era of electronics, precious gold is endlessly being dumped as a solid waste (i.e., e-waste)/or in streams (primary or secondary gold containing effluents released during gold ore processing in form of leachate or spent liquors) remains unexploited (Cucchiella et al., 2015; Garlapati, 2016). The gold concentration in e-wastes are mostly of low grade value, and are difficult to retrieve using the conventional chemical methods such as cyanidation or chemical leaching (Scott, 2014). Since past few years, biological (i.e., microbe-mediated) gold mining has emerged as a suitable choice to biotreat the low-grade auriferous ores encased inside the e-wastes (Kucuker and Kerstin, 2018). Various biohydrometallurgical methods has found effective gold bio-recovery such as, bioleaching (Yuan et al., 2018), biooxidation (Huang et al., 2019a), bioprecipitation (Achinas et al., 2017), bioaccumulation (Ilyas and Ilyas, 2018) and microbial electrochemical technologies (Fig. 2) (Nancharaiah et al., 2016). Some highly promising microbe driven technologies of known process like biofiltration or gas biofiltration are yet to be implicated fully for precious metal ion recovery (Cheng et al., 2016; Srivastava and Majumder, 2008; Yang et al., 2010a, 2010b). Using specialised biofilters can be designed for the specific recovery of the metal ions which can pave a way for fast and efficient gold recovery (Yang et al., 2018).

Here, biological gold biomining refers to the microbe assisted mining of gold, in which microorganisms or its components are used to extract metal ions from low grade ores or wastes. It has already been implicated industrially to process sulfidic and uranium ores, but its promising potential against other metals has been confined to lab scale only, and yet to be applied industrially. On the other hand, biohydrometallurgical methods are microbe-based metal extraction processes in which microbial metabolic agents (oxidants and/or acids) are exploited as the chief metal extractors to recover the precious metal ions from low-grade ores or e-wastes. These methods are more economical and leaves minimal environmental imprint, and its unique ability to harness metal ions from lower-grade ores or e-wastes. These methods are more economical and leaves minimal environmental imprint, and its unique ability to harness metal ions from lower-grade ores or e-wastes sets it apart from the conventional methods and make it more appealing. For instance, process of bioleaching is a widely used metal solubilization method used to retrieve the precious metal ions from sulfidic low-grade ores or e-wastes by using unique microbes (e.g., cyanogens) releasing the unique bioleaching/solubilising agents into aqueous solutions, on the other hand, bio-oxidation method of metal
recovery is achieved by microbes by merely oxidizing the metals entrenched surfaces (e.g., used industrially as post processing method to recover gold in large-sized stirred tanks). Microbe based bioreactors can cost-effectively treat the moderate to high volumes of metal ion concentrates while allowing the flexible and controlled process operability at diverse parameters (like pH, temperature) during reactor operation, thus rendering improved metal biomining performances, which can further be boosted by choosing appropriate configurations suitable for the bioleaching or other biominning processes (Acevedo et al., 2000). The process of biomineralization utilizes the microbial ability to mineralize the metal ions (inside or outside its cells) as nanoparticles (known for its wide range of applications in the field of nanotechnology/medicine, effluent treatment and bioremediation) from metal concentrates or metal embedded earth surface, while bioadsorption involves only the attachment of the microbial cells on the surface of the metal ore entrenched surface/liquid concentrates. Moreover, bioprecipitations involves the microbial ability to precipitate or vomit out the metal particle inside or outside its body as a response to the metal toxicity in solid form by forming metal ion complexes to minimize the toxic effect, on the other hand, bioaccumulation is a microbe assisted metal accumulation method where microbe uses its metabolic machinery to bioaccumulate the metal ions inside its body (Fig. 3, Diep et al., 2018). These biomining methods are driven by gold harvesting microbes, which are either isolated from the gold enriched areas and domesticated for its ability to withstand high gold pressures or are bioengineered strains with exceptional gold retrieval efficiency. For instance, thermophilic and hyperthermophilic bacteria/archaea i.e., Thermotoga maritime and Pyrobaculum islandicum can precipitate the gold in gold-bearing sinters forms, while another bacterial type, sulphate-reducing bacteria i.e., Desulfuviibrio spp. Deltaproteobacteria spp. and Firmicutes spp. can lead to gold-bearing sulphide formation (Winch et al., 2009). Also, the chemolithotrophic microbes, such as iron- and sulphur-oxidizing bacteria (i.e., Acidithiobacillus ferrooxidans, A. thiooxidans) biomineralize the gold-hosting sulphide minerals by synthesizing the gold oxidising/complexing agents (i.e., thiosulphate). Role of various gold bioaccumulating microbes and their role in biogeochemical cycling of gold has recently been reviewed (Shuster and Reith, 2018). These microbes can exceptionally endure excessive gold toxicity due to their ability to cause reductive precipitation of gold by its complexation (using gold-complexing ligands such as cyanide, humic acid, thiosulfate, bisulfide and chloride) or ejecting toxic gold using the microbial P-type ATPase efflux pumps/or membrane vesicles to detoxify themselves immediately (Fig. 4, Reith et al., 2007a). Moreover, microbes release several secondary metabolites and extracellular polymers as a response to gold toxicity to help them sequester gold, thereby ensuring their survival in toxic auriferous environments.

Complex biominning processes involve various microbial communities with different compositions and functions. Ease of applicability of bio-hydrometallurgical techniques has potential to realize the long term gold mining goals, but changing dynamics of gold metabolising mechanisms in different gold collecting microbes isolated from different gold mining sites is still a challenge which remains to be understood (Savvaidis et al., 1998). For instance, dominant strain types vary from one bioleaching system to another, and not necessarily the same microbial consortium duplicate itself in different biosystems. The technological breakthroughs in field of molecular genetics, genomics, proteomics and metabolomics have improved our understanding of the gold-microbe interaction mechanisms at gene level (Tay et al., 2013). For example, the
transcriptional regulator genes of MerR family helps microbes to sense the auriferous stimuli/or oxidative stress as a response to gold toxicity, whereas gol locus imparts the gold resistance to them. Also, the genes associated with GolS family dictates the metal-ion selectivity in the gold accumulating microbes (Checa et al., 2007). Scope of novel approaches such as microbial electro-metallurgy and bio-electrochemical approaches in metal recovery has potential to make the process of the gold bio-recovery highly specific and efficient (Dominguez-Benetton et al., 2018; Huang et al., 2019b). This review aims to discuss the current promising microbes-mediated approaches used to bio-mine gold from e-wastes and low-grade ores. At present, conventional chemical methods are still the chief method of gold mining due to their high recovery rate within short recovery time. The first part of this review discusses various biomining methods along with challenges and opportunities associated with each method. While the later part of review discusses about resilience of the microbial pathways and how it differs microbe to microbe depending on its site of isolation and how it dictates our choice of microbial stain selection for various biomining sites. Scope of novel molecular tools in designing commercial genetically modified organism capable to withstand harsh conditions with improved gold recovering potential within least possible time has also been discussed in this review.

Existing potential of biomining industry in facilitating the extraction of valuable gold from low-grade ores containing wastes or metal waste infested soil can be achieved by integrating various biomining methods (run by genetically advanced microbes) with existing technologies to fully exploit the natural resources of gold ore irrespective of its grade type.

1. Microbes assisted gold biomining

The microbes assisted metal bio-recovery using biomining has successfully been implicated to extract several important metals (e.g., gold, copper, uranium, zinc) in industrial operations (Dong et al., 2013; Fowler and Crundwell, 1999; Munoz et al., 1995). Commercial implementation of the microbial assisted gold recovery has commercially been applied using heaps, dumps and stirred tank bioreactors (Jerez, 2017). Such biotechnological biomining methods contribute a significant percentage (several hundred thousand tons) of bio-mined gold annually. Since last few decades, the use of bacteria (Romero, 2018), algae (Chakrabirty et al., 2009) and fungi (Mata et al., 2009) for gold biomining has increased (Table 1). The microbial consortium that chiefly contains chemolithotrophs and mixotrophs (e.g., Acidithiobacillus ferrooxidans/thiooxidans) have found to be efficient in gold recovery due to their ability to utilize the iron- or reduced inorganic sulphur compounds as source of energy (Mubarok et al., 2017). Also, sulphur reducing bacteria (e.g., Desulfovosporinus spp.) has been found to immobilise gold ions along with copper and iron under low pH and anoxic conditions (Winch et al., 2009). Despite the high economic viability and sustainability of the gold bio-recovery
methods, excessive usage of some microbes (e.g., acidophiles) can also be hazardous to humans and environment due to their ability to solubilise or mobilise various non-targeted metal ions and bioleach them into our water bodies (Brierley, 2008). So, carefully planned implementation strategies are required before taking these bioprocessing methods to commercial scale while considering all-pros and cons.

2. Various biomining methods of gold recovery

2.1. Bioleaching

Microbes mediated mineralization of gold is an environmentally sustainable method for gold recovery. The gold can be bioleached from aqueous gold ore concentrates of the wasted electronics (which otherwise have hazardous effect on human health and environment) by applying the cyanogenic microbial culture. Some microbes acts as a biogenic lixiviant for gold bio-recovery due to their unique ability to produce the bioleaching agents such as cyanide as a secondary metabolite (Tay et al., 2013). For example, the cyanogenic bacteria Chromobacterium violaceum can mobilizes the gold ions by secreting cyanide as a secondary metabolite into the solution which leads to the soluble gold-cyanide complex formation. Similarly, bacteria Delftia acidovorans produces a secondary metabolite namely ‘delftibactin’ which help it in mineralizing/or precipitating the gold. The poor gold removal efficiency of this method is still a major challenge that need to be overcome, as concentration of lixiviants produced by the microbes are not sufficient enough to sustain the high scale of gold recovery rate (Pant et al., 2012). Several strategies has been used to overcome these challenges such as pre-treatment of the gold containing e-waste to enhance gold ion exposure (while removing the other competing metal ions), genetically modifying the microbial strains to adapt them to endure high pH conditions and engineering microbial metabolism by incorporating extra copy of cyanide producing operon or by decoupling of cyanogenesis from quorum control to make them produce more lixiviant cyanide. These strategies are noted to significantly increase the gold recovery rate from 7.1% to 30% using 0.5% (W/V) of pulp density of the gold concentrates (Das et al., 2017). Extracting gold using the biogenic cyanide is an eco-friendly alternative which often termed as alkaline bioleaching or heterotrophic bioleaching. Additionally, the major bacteria known for HCN production are Pseudomonas aeruginosa, Pseudomonas plecoglossicid, Chromobacterium violaceum and Pseudomonas fluorescens. Also, fungi Marsmius oreades is reported to produce HCN. The enzyme HCN synthase is responsible for the production of biogenic HCN in different microbes, which is synthesised between the exponential and stationary phase of microbial growth and ultimately detoxified at the end of stationary phase by converting it into β-cyanoalanine (The microbes assisted metal bio-recovery using biomining has successfully been implicated...
<table>
<thead>
<tr>
<th>Gold retrieving microbes</th>
<th>Process</th>
<th>Gold removal efficiency (%)</th>
<th>Recovered gold concentration</th>
<th>pH</th>
<th>Temp (°C)</th>
<th>AuNP size (nm)</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. oneidensis MR-1 (anoxic)</td>
<td>Bioelectrosorption</td>
<td>88.5</td>
<td>26.05–70 mg Au(III)/g cell dry weight/hr</td>
<td>1</td>
<td>37</td>
<td>5-10 (without H₂), 30-100 (with H₂) (Nakajima, 2003; De Corte et al., 2011; Feng et al., 2015; Romero, 2018)</td>
<td></td>
</tr>
<tr>
<td>C. metallidurans CH34 (oxic)</td>
<td>Biosorption</td>
<td>92.8</td>
<td>26.78 mg</td>
<td>5</td>
<td>28</td>
<td>15–22</td>
<td>Romero (2018)</td>
</tr>
<tr>
<td>Shewanella halotisa</td>
<td>Biosorption</td>
<td>99</td>
<td>-</td>
<td>5</td>
<td>10–30</td>
<td>-</td>
<td>Zhu et al. (2016)</td>
</tr>
<tr>
<td>Chromobacterium violaceum</td>
<td>Biosolubilisation</td>
<td>83</td>
<td>0.34 mg/L</td>
<td>8.5</td>
<td>-</td>
<td>-</td>
<td>Campbell et al. (2001)</td>
</tr>
<tr>
<td>Pseudomonas plecoglossicida</td>
<td>Bioleaching</td>
<td>69</td>
<td>500 mg Au/L</td>
<td>6.5</td>
<td>-</td>
<td>-</td>
<td>Faramarzi and Brandi (2006).</td>
</tr>
<tr>
<td>Heterotrophic bacteria</td>
<td>Biomineralisation</td>
<td>80</td>
<td>35 mg/L or 1134 ng/g to 2930 ng/g Au</td>
<td>-</td>
<td>25</td>
<td>-</td>
<td>(Reith and McPhail, 2006, 2007)</td>
</tr>
<tr>
<td>Delftia acidovorans and Cupriavidus metallidurans</td>
<td>Bioleaching</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10–200</td>
<td>-</td>
<td>Reith et al. (2010)</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>Biosolubilisation</td>
<td>-</td>
<td>-</td>
<td>7.4</td>
<td>RT</td>
<td>4.5–9</td>
<td>Feng et al. (2015)</td>
</tr>
<tr>
<td>Plectonema boryanum</td>
<td>Bioaccumulation</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>25</td>
<td>60–6e+9</td>
<td>Lengke et al. (2006)</td>
</tr>
<tr>
<td>Fucus vesiculosus;</td>
<td>Biosorption</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>Mata et al. (2009)</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>25</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rhizoclonium hieroglyphicum and Lyngbya majuscula and Spirulina subsalsa</td>
<td>Biosorption or bioaccumulation -</td>
<td>90–100</td>
<td>3.28, 1.93 and 1.73 mg/g</td>
<td>6</td>
<td>-</td>
<td>15–30</td>
<td>Chakraborty et al. (2009)</td>
</tr>
<tr>
<td>Spirulina platensis</td>
<td>Biosorption</td>
<td>-</td>
<td>-</td>
<td>5.6</td>
<td>37</td>
<td>6–10</td>
<td>Govindaraju et al. (2008)</td>
</tr>
<tr>
<td>Leptolyngbya tenuis, Coleofasciculus chthonoplastes, and Nostoc ellipso sporum</td>
<td>Bioaccumulation</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>Parial et al. (2016)</td>
</tr>
<tr>
<td>Arthrobacter spp. 61B and Arthrobacter globiformis 151B</td>
<td>Bioaccumulation</td>
<td>-</td>
<td>-</td>
<td>7–12</td>
<td>20–30</td>
<td>8–40</td>
<td>Kalabegishvili et al. (2012)</td>
</tr>
<tr>
<td>Lysinibacillus sphaericus</td>
<td>Biosorption</td>
<td>75–95</td>
<td>-</td>
<td>30</td>
<td>50–100</td>
<td>-</td>
<td>Bustos et al. (2018)</td>
</tr>
<tr>
<td>Stenotrophomonas spp.</td>
<td>Biosorption</td>
<td>91</td>
<td>0.040 mmol/g</td>
<td>2.0</td>
<td>25</td>
<td>24.7–31.4</td>
<td>Huiping et al. (2013)</td>
</tr>
<tr>
<td>Corynebacterium glutamicum</td>
<td>Biosorption</td>
<td>-</td>
<td>-</td>
<td>5.5</td>
<td>25</td>
<td>6 × 10⁴</td>
<td>Park et al. (2012)</td>
</tr>
<tr>
<td>Sargassam b</td>
<td>Biosorption</td>
<td>91.4</td>
<td>-</td>
<td>2</td>
<td>RT</td>
<td>20–200</td>
<td>Sathishkumar et al. (2010)</td>
</tr>
<tr>
<td>Chromobacterium violaceum, Delftia acidovorans</td>
<td>Bioleaching</td>
<td>11–36.5</td>
<td>-</td>
<td>9–10</td>
<td>30–40</td>
<td>31 × 10⁹–75 × 10¹</td>
<td>Das et al. (2017)</td>
</tr>
<tr>
<td>Chromobacterium violaceum pBAD</td>
<td>Bioleaching</td>
<td>24.7–36.4</td>
<td>-</td>
<td>40</td>
<td>-</td>
<td>-</td>
<td>Das and Ting (2017).</td>
</tr>
</tbody>
</table>

RT: room temperature.
to extract several important metals (Knowles and Bunch, 1986)).

The gold solubilisation can be expressed as following reactions,

$$4Au + 8CN^- \rightarrow 4Au(CN)_2^- + 4e^-$$  \hspace{1cm} (1)

$$O_2 + H_2O + 4e^- \rightarrow 4OH^-$$  \hspace{1cm} (2)

$$4Au + 8CN^- + O_2 + 2H_2O \rightarrow 4Au(CN)_2^- + 4OH^-$$  \hspace{1cm} (3)

Moreover, bioleaching of gold can be accomplished using the spent medium containing microbial cell free metabolites (e.g., cyanide lixiviant). In this process, microorganisms are grown till its late logarithmic or early stationary phase after which microbial mixture is made cells free by through centrifugation (which otherwise proceed HCN conversion to β-cyno-alanine), thus ultimately left with the spent medium with lixiviant (and more oxygen) only with multiplied ability to bioleach gold. For instance, gold bio-recovery from e-waste concentrate (0.5%, W/V pulp density) has found to increase gold biorecovery potential from 11% to 18% when cyanogenic Chromobacterium violaceum spent medium was used instead of whole cell suspension. Although the e-waste was pre-treated with nitric acid which might also have contributed to reduce the other competing metal ions by dissolving them, which otherwise compete for cyanide ions. Interestingly, pH of the spent medium play an important role in steering its gold extraction potential, which nearly can double the gold recovery rate at optimum pH as it ultimately shifts chemical reactions to favourable equilibrium that lead to more cyanide production (Natrajan and Ting, 2013). High pulp densities of gold containing electronic concentrate often proves highly toxic to microbes, so decrease in concentrate’s pH can be observed which adversely affects the microbial growth and thus leading to reduced overall gold bioleaching efficiency (Natarajan and Ting, 2015). So, high gold recovery from high pulp density concentrates can be achieved by balancing the high pH of the concentrates that favours cyanide ion formation required for high gold complexation. Utilising the metabolic and proteomic engineering technologies to construct enhanced bioleaching microbes capable to overproduce the lixiviant and thus helping in extracting more gold, for instance, the cyanide lixiviant production rate was found to surge by 70% in a metabolically-engineered Chromobacterium violaceum with twice gold retrieval efficiency than its wild type strain (Tay et al., 2013). Additionally, the spent medium (obtained from bacterium Delftia acidovorans after incubating for 16–20 hr at 30°C) assisted bioleaching gold form solid wastes with initial pH adjustment has led to the 30% of the gold removal rate (Das et al., 2017). Decoupling the bacterial growth with the gold complexation helps in attaining peak cyanide concentration in turn significantly enhancing the high gold recovery.

The thiourea tolerant microbes (such as Fe-oxidizing bacteria/archaea) mediated thiourea bioleaching of gold helps in facilitating the gold dissolution with minimal reagent usage, thereby solving the major limitation associated with chemical cyanidation. For instance, Acidiplasma spp. Fv-Ap strain were found to dissolve 98% of gold from aqueous e-wastes solution due to its ability to regenerate Fe$^{3+}$ which reduces the reagent concentrations usage to 1 mmol/L of Fe$^{3+}$ and 10 mmol/L of thiourea Eq. (3) and Na$_2$SO$_3$ can help in preventing the decomposition rate of thiourea by 62% in aqueous solution by reverting the thiourea oxidation reaction (Eqs. (3) and (4)) which otherwise get oxidized to formamidine disulphide which slows the process of bioleaching (Rizki et al., 2019). Under acidic conditions, the gold’s dissolution leads to form a stable Au-thiourea complex and thiourea immediately said to get oxidized into formamidine disulphide as shown,

$$\text{Au} + 2\text{CS(NH$_2$)$_2$} = \text{Au(CS(NH$_2$)$_2$)}^{2+} + e^- \hspace{1cm} (E^0 = -0.38 \text{ V})$$  \hspace{1cm} (4)

$$2\text{CS(NH$_2$)$_2$} = (\text{NH$_2$)$_2$CSSC(NH$_2$)$_2$}^{2+} + 2e^- \hspace{1cm} (E^0 = -0.42 \text{ V})$$  \hspace{1cm} (5)

$$4\text{Fe}^{2+} + \text{O}_2 + 4\text{H}^+ = 4\text{Fe}^{3+} + 2\text{H}_2\text{O}$$  \hspace{1cm} (6)

$$\text{NH}_2(\text{NH})\text{CSSC(NH)$_2$} + \text{SO}_4^{2-} + \text{H}_2\text{O} = 2\text{CS(NH$_2$)$_2$} + \text{SO}_4^{2-}$$  \hspace{1cm} (7)

Generally, the cyanogenic microbe, Chromobacterium violaceum in auriferous soil are reportedly known to possess quorum-sensing-regulated cyanide production, which further arm this microbe with ability to dissolve gold (McGivney et al., 2018). Additionally, using potential high-throughput strategies to metabolically engineer the microbial strain to surge their biogenic-cyanide producing ability (which increases the lixiviant availability) also significantly increases the Au recovery (Yuan et al., 2018). The genetically engineered strain of Chromobacterium violaceum has been to synthesize more cyanide than its wild type has been reported to significantly enhance its gold dissolution potential (Tay et al., 2013). In addition, the Gold bioleaching efficiency of metabolically engineered Chromobacterium violaceum pBAD has reported to improve from 24.7% to 36.4% by pre-treating the aqueous gold solution with a mixture of hydrogen peroxide and sulfuric acid which help in removing the excessive copper which otherwise interfere with the gold extraction process (Das and Ting, 2017). However, adsorbing and binding ability of microbial extracellular polymeric substances has reportedly been found to hinder the bioleaching process. Polarity of microbial surface is dictated by presence of certain functional groups (such as carboxyl, hydroxyl, and amine groups) which decide the fate of microbe-gold interactions, which ultimately steer outcome of any bioleaching process. But integration of the biological process with chemical agent like polyvinylpyrrolidone was found to minimize these interactions by moderately damaging the microbial cells which help in minimizing the diffusion control between Pseudomonas fluorescens and metal ions (e.g., integrated PVP-bioleaching has been successfully been implicated for silver retrieval, which has increased recovery rate by 1.8 times) (Pant et al., 2012). Employment of similar integrated approach has been suggested to achieve the maximal economic benefits form refractory ores, where biological reactor (capable to propagate micro-cavitation with oxygen) was found to reduce the leaching time for conventional cyanidation process and to enhance the gold retrieval to 10%–15% within 2 hr (Mbayo et al., 2019).
Biofilms deposition is prevalent phenomenon on or inside the auriferous soils or sediments. The synergistic interactions between different microbial communities of the biofilm matrix directly influence the adhesion efficiency and bioleaching potential of its individual microbial subunits. For instance, an acidophilic bacterium (Leptospirillum ferrophilum) to acidophilic archaeon (Ferromicrobium acidiphilum) interactions during pyrite bioleaching has found to negatively affect the former’s ability to attach with pyrite, thereby lead reduced overall bioleaching potential (Reith et al., 2010). In contrary, interactions between two bacteria, F. acidiphilum with S. thermosulfidooxidans have increased the overall bioleaching potential in another study, however, these interactions seem to have no correlation with enhanced cellular attachment to metal ions (like the pure microbial cultures) (Maulani et al., 2016). Similarly, fungal strain, Aspergillus niger MXPE6 and Aspergillus niger MX7 has collectively been found to bioleach the 28%–87% of gold from e-wastes then individual counterparts, thereby suggesting the fungal consortium based bioleaching is equally promising for metal recovery than its bacterial counterparts for the gold biorecovery (Madrigal-Arias et al., 2015). Improvement of the metal recovery efficiency by means of fungal metabolism has already been discussed elsewhere (Madrigal-Arias et al., 2015). Collectively, bacteria and algae clearly have the potential to lay the foundation of the next generation biohydrometallurgical applications.

Interestingly, excellent gold capturing ability of mixotrophs (mixed microbial consortiums capable to retrieve carbon and energy using either auto- or heterotrophy) has also been investigated by few researchers, which have confirmed its unique ability to withstand wide range of pH, its ability to use organic/or inorganic matter and iron/or sulfur as the source of carbon and electron respectively. For example, the mixotrophic iron- and sulfur-oxidizing bacterial strains (SKC1 and SKC2) was found to significantly improve the gold extraction rate by increasing the biooxidation of refractory gold concentrates during cyanidation at neutral pH. Mixotrophs usually secrete the extracellular polymeric substances (EPS) as construction material of its settlements on any targeted metal surface. It helps them in establishing easier iron or sulfur-to-EPS anionic complexation, which protect them by averting the sulphide mineral passivation during iron and sulfur ion precipitation on mineral surface, thus ultimately enhance the cyanidation with cyanide ions to form thiocyanate and ferro-cyanate ions (Mubarok et al., 2017). However, the functional role of synthesized EPS and dissimilarity of the gold fixing mechanism in different mixotrophs are yet to be fully understood and calls for further research. However, application of bioleaching is still in its infancy and few limitations yet remained to be resolved. Major issue associated with bioleaching the gold from e-wastes is lack of an efficient method of separating the non-metallic ions from precious gold ions during the industrial scale production. Inefficient separation often proves detrimental for microbial growth thereby slowing down the bioleaching with delayed gold recovery. So, recovering the precious gold from the auriferous waste will require integration of two or more technologies (i.e., corona-electrostatic separation) with bioleaching method (i.e., hybrid technologies) to overcome interference caused by non-metallic ions which destroys the bioleaching processing of the gold (Jujun et al., 2015). Moreover, using the lab-scale gold harvesting strains instead of commercialized and optimised cyanogenic strains for gold retrieval is another reason behind low efficiency of bioleaching at industrial scale. Also, only few researchers have given a detailed overview of bioreactors-based bioleaching and its optimisation to recover maximum gold out of auriferous gold ore or solid electronics waste. So, future research should create bioreactors with advanced bioleaching efficiency at pilot scale and also should study the bioleaching potential of various bioreactor designs to ensure maximum rate of gold bio-recovery and minimize the over-long gold production cycle. Effect of various physiochemical parameters on microbial dynamics need to be studied at molecular level to make knowledgeable decisions based on gained refined perspective about the microbe-gold interactions during bioleaching. Such understanding of microbial components and functionality at gene level will help in improving the efficiency and rates of bioleaching system. Lack of gold solubilisation over pH range of pH 2–10 can be improvised by engineering the gold harvesting microbial strains capable to dissolve the gold over wide range of pH.

2.2. Biominalization

Biological processes (microbial weathering and bioleaching) frees gold from its mineral forms (pyrite or arsenopyrite), integrity of which was sustained by gold-complexing ligands (thiosulfate, amino acids, and cyanide) mineralisation. These can only initiate the gold’s solubilization via complexation promoted by their oxidation. Although, the toxicity associated with the gold complexes leads to intracellular oxidative stress and enzymatic dysfunction, but the syntrophic mode of coexistence between the multi microbial biofilm communities help one-another to endure them (Rea et al., 2016). The deposition of biofilm on gold’s surface helps in driving the gold solubilization/or re-concentration which is a mean by which microbes detoxify itself to get rid of any intra- or extracellular toxicity emerged out of the presence of excessive gold. Involvement of such metallophillic bacterium biofilms in detoxification of Au-complexes has been validated (assisted by metal-resistance gene clusters which promote cellular defence) and established its role in promoting Au biominalization by accumulating Au(III)-complexes from solution or near-surface environments via energy-dependent reductive precipitation (Fairbrother et al., 2013). Mechanism of gold mineralization has been studied using a metallophillic bacterium, Cupriavidus metallidurans along with the gold regulated gene expression pattern which revealed the concentration of Au(III)-complexes at cellular level (Reith et al., 2009). The cellular defensive mechanism of this microbe activates the gold resistances gene clusters (or operon) which help in inducing intracellular oxidative stress inside the cell as a response to the gold toxicity to initiate various detoxifications processes, such as efflux of gold outside the cell, intracellular reduction or Au-complexes methylation (Fairbrother et al., 2013). Thus, knowledge of such specific genetic responses of various gold precipitating microbes could help us design highly advance and sensitive tools for bio-exploration and bioprocessing.
Recently a study has validated the role of bacteria *Cupriavidus metallidurans* in organo-platinum compound formation/and transport followed by its immobilisation in natural systems (that leads to dispersion halos formation) where it detoxifies itself by shedding outer membrane vesicles accumulated with metallic, colloidal platinum (Campbell et al., 2018). Unique metal-detoxification pathway inside this microbe helps it to withstand close proximity of toxic transition metal ions, and prevents synergistic toxicity caused by two or more metal ions like Cu and Au complexes, where Au(I) shut down its efflux system (CupA) against excessive cytoplasmic Cu(I). Thus, CopA oxidase oxidises the periplasmic Au(I) and Cu(I) ions into Au(III) and Cu(II) to prevent it from further cytoplasmic uptake, thereby reducing subsequent synergistic toxicity level by directly reducing Au(III) to periplasmic Au(0) nanoparticles (Bütof et al., 2018).

Similarly, another microbe, *Delftia tsuruhatensis* GX-3 has been isolated which can extracellularly bio mineralize gold from heavy metal contaminated paddy soil. Researchers proposed that this bacterium might be responsible for gold bioaccumulation and bio-precipitation by sulphur-reducing bacteria in given natural environment. An extracellular solute-binding protein and/or porin was reportedly said to involve in gold nanoparticles synthesis as well as in detoxification, which mediates the electrons transport to Au precipitation (Li et al., 2018). In addition, correlation between degree of Au-particle transformation/or Au-cycling with biofilm community composition has been revealed, where metal-resistant Au-cycling taxa (Pseudomonas, Leptothrix, Acinetobacter, Rhodoferax and Geobacter) was found to cause Au transformation (Rea et al., 2018). Also, the EPS surrounding the bacteria *Escherichia coli* also play a critical role in mediating the extracellular biomineralization of gold into nanoparticles form (95.2%) as removal of which has role in mediating the extracellular biomineralization of gold surrounding the bacteria *Escherichia coli* of gold.

**2.3. Bioprecipitation or bioreduction**

Biological process of bio-precipitation is another alternative to physical methods to recover gold. Alfalfa (*Medicago sativa*) plant biomass can effectively bind/and reduce gold from Au(III) ion containing solutions into retrievable gold(0) nanoparticle at wide range of pH implying the covalent nature of gold ions binding than electro-static interactions (Achinias et al., 2017). Reductive bio-precipitation is an enzymatically assisted mode of precipitating the precious metal by turning its positive valence state to zero valence state. Such metal bio-reduction can be a result of direct or indirect (through electron mediators) contact to microbial cell surface. Secondary metabolites (also called metallophores) bestow the survival advantage to metal tolerant microbes and ensures its binding to the metal surface (Johnston et al., 2013). For instance, the *Delftia acidovorans* produces such metallophore which shield it against gold toxicity and further helps gold biomineralization. Metal bio-reduction process encompasses several multi-electron transfer pathways, which are either not discovered or yet to be fully understood. Enzymatically catalysed bioreduction of Au$^{3+}$ depends on type of electron donor type (e.g., hydrogen) used in bioprecipitation. Process of reductive bio-precipitation of gold using the iron reducing microorganism or archaea differs significantly from process of gold bio-accumulation (İşildar, 2018). A microbe, *Delftia acidovorans* secretes a chemical, delftibactin, which helps it to bio-precipitate gold from the cyanide solution. Also, further genetically manipulations of this bacteria to stimulate 'delftibactin' production can significantly improve its gold retrieval efficiency (Das et al., 2017). The hybrid mechanism of gold bioaccumulation can be explained by microbially enhanced chemisorption of metal ions via its intercalation with pre-formed biogenic gold crystals. Bioaccumulation of the gold ions are more of adsorptive nature than intracellular, so more understanding of the gold ion adsorption based on surfaces of gold ions of different oxidation states can help to enhance the efficiency of the microbes as the gold bio-accumulator. Although, very few studies have targeted the microbial mediated bioaccumulation based on different reduced forms gold, but we can extrapolate its mechanism from behaviour of other elements closely related to gold. Thorough researches are needed which solely targets the microbial mechanisms which help in counteracting with gold toxicity and bio-accumulation, thereby might resolve the existing issues associated with the use of live cells for gold ion bioaccumulation.

**2.4. Biosorption**

Biosorption is a green route to recover critical metals using the low-cost bio-sorbents. Bio-sorbents for gold removal include bacteria (Bustos et al., 2018), fungi (Sathishkumar et al., 2010), and algal (Mata et al., 2009) biomass. Biosorption is considered as a promising method of gold recovery because of its cost effectiveness, eco-friendliness, high metal binding, great settleability, biosorbent regenerability, high efficiency in diluted effluents and ease of separation from metal concentrates.
The unique ability of the inactive/dead microbial biomass to attach to the metal ions via various physicochemical mechanisms makes it an excellent tool to biomining the gold by biosorption/or bioadsorption. The biosorbtion to metal ion interaction depends on the chemical make-up of the microbial cells (Volesky and Holan, 1995). The biosorption mechanisms is a combination of two more chemical processes (like electrostatic interaction, ion exchange, metal ion complexation/or chelation or micro-precipitation) occurring either intra/or extracellularly, which ultimately dictate the fate of any metal biosorption (Fig. 3) (Diep et al., 2018). Detailed microbial mechanicistic of microbes especially gram positive and gram negative bacteria. Presence of specific functional groups (such as carboxylates, sulfates, phosphates, amino-polyasacharides, imidazole, sulphhydril, thioether, phenol, carbonyl, amide and hydroxyl) on microbial cell wall assign it a unique fingerprint, which allows or rejects its adsorption to specific metal ions, thus dictates feasibility of a certain adsorptive removal possibility for metal ions of interest (Wang and Chen., 2009; Sathishkumar et al., 2010). Applicability of the biosorbsornts (like bacteria, fungi and algae) in metal removal has already been elaborately explained in terms of their structure, biosorption potential, modification, regeneration/reuse and development of novel biosorbents for its future potential application in technologies based on biosorption or bioadsorption to further enhance their sorption capacity (Wang and Chen, 2009). The gold absorption potential of a gram-positive bacillus Lysinibacillus sphaericus have been isolated from an active gold mine and were applied to remediate the contaminated matrices or gold containing soils with 75–95% of gold recovering efficiency (within 50 hr) (Bustos et al., 2018). Mechanism of gold biosorption by microbes has been discussed previously (Kuyucak and Volesky, 1989b) along with sequestered gold elution processes (Kuyucak and Volesky, 1989a). Microbial biosorption capacity has also been exploited in terms of its ability to recover gold. Bioadsorption using a magneto-tactic bacterial isolate, Stenotrophomonas spp. (506 mg/g) to recover Au(III) has been investigated with 91% of Au recovery on biomass (0.8 mol/L of thiourea as desorbant) (Huiping et al., 2013). Moreover, excellent gold absorptive ability of Magnetospirillum gryphiswaldense MSR-1 helps this microbe to biosynthesize different sized gold nanoparticles intracellularly, size of which directly influenced by pH and initial concentrations of gold during experiment (Cai et al., 2011). Also, Corynebacterium glutamicum is known for its ability to firm gold-loaded fibres, gold Au(I) uptake ability of which can maximized (to 421.1 mg/ g at pH 5.5) by treating it with suitable chemicals (e.g., poly-ethylenimine) (Park et al., 2012). Integration of biosorptive method with other methods (such as biocrystallization and pyro-crystallization) can increase the gold biosorption potential of Sargassum biomass to 90% and shortens the gold recovery time (within 15 min) (Sathishkumar et al., 2010). Using microbial biomass (living or non-living) based biosorption can prove to be an effective strategy to recover gold from aqueous solutions, however further researches are required to validate it (Cai et al., 2016). Out of living and non-living microbial biomass assisted biosorption, where former is non-responsive to toxic metal ions and not requires nutrients replenishment, but its passive absorbability limits it to limited area of microbial surface only, whereas later has access to its internal metabolic machinery with evolved resistivity to metal ions (metal detoxification) which makes it efficient mean of the gold bio-recovery, but regularly requires nutrient supply to sustain its growth (He et al., 2018). Engineering process application potential of this biomining process can be improved by increasing the metabolism-independent uptake of the gold by microbes by genetic engineering tools, and to conduct studies to optimise the functionality and effect of genetically modified strains on gold biosorption. Knowledge of recurring changes in functional groups during gold ion (before and after) biosorption can impart valuable insight into finding characteristic novel groups essential for binding of precious metals (i.e., gold or silver).

2.5. Bioaccumulation

Potential application of metal bioaccumulation has increased substantially. The gold bioaccumulation can be achieved by using bacteria (Nakajima, 2003), algae (Mata et al., 2009), fungi (Moore et al., 2008) or plants. The bacterial mechanism of gold bioaccumulation from gold solutions has been studied for bacteria previously (Fig. 3, Diep et al., 2018). Algal biomass is known as a promising bioaccumulator of gold and other metal ions due its efficient and selective recovery potential. Previously, mechanism of algal-Au(III)/or (I) interaction has been investigated for Chlorella vulgaris and speculated about the importance of competing ligands (such as mercaptoethanol, cyanide and thiourea) on its gold absorbability and binding efficiency in sodium tetrachloroaurate(III) or sodium gold(I)-thiomolate containing aqueous solutions. Presence of hydroxyl groups on algal (Fucus vesiculosus) biomass surface (made of polysaccharides) reportedly help it in microprecipitating the gold (in nanoparticle form) from e-waste leachates. Gold biosorption and bio-reduction ability of due to which (Mata et al., 2009). The brown algae (such as Ecklonia cava, Cystoseira baccata) known for its great ability to uptake various heavy metals (Venkatesan et al., 2014; González-Ballesteros et al., 2017). Presence of the carboxyl groups on brown algal cell wall along with its ability to secrete a polysaccharide, called Fucoids assist it to synthetize the gold nanoparticles intracellularly (Venkatesan et al., 2014). The microbial tendency of metal-uptake has fascinated the researchers since last few decades, various efforts to explore its potential has been made. The gold cations uptake by Sargassum biomass was reportedly involve reduction mechanism, where its highly reducing component i.e., tannin present in its cell wall assists in gold reduction. The bioaccumulation potential of few algal species such as Rhizoclonium hieroglyphicum (3.28 mg/g) and Lyngbya majuscula (1.93 mg/g) and Spirulina subsalsa (1.73 mg/g) have even more promising, due to ability of their biomass to visibly indicate the gold reduction i.e., Au(III) to Au(0) at intra- and extracellular level by changing its colour (to purple) (Chakraborty et al., 2009). Algae and blue-green algae-assisted recovery of gold nanoparticles and its applicability has been widely been reviewed (Khan et al., 2019). In addition, Spirulina platensis can effectively mediates the extracellular synthesis of gold and silver nanoparticles simultaneously (at 37°C and pH 5.6) (Govindaraju et al., 2008). Similarly, using the cyanobacterial
biomass can be an effective strategy to retrieve gold (20 mg/100 mL of 15 mg/L Au(III)solution at pH 5), which respond to metal stress showing escalation at enzymatic level followed by visible decline in pigmentation. Additionally, variation in protein content at cellular level (another cyanobacterial response against gold toxicity) is said to be responsible for the bioreduction gold nanoparticles (Parial et al., 2016). Microalgal biocomponent based recovery of gold nanoparticles have also been reviewed elsewhere (Shankar et al., 2016). Extracellularly gold can be retrieved from gold chloro-aurate solution using Arthrobacterial strains (like Arthrobacter spp. 61B and Arthrobacter globiformis 151B) (Kalabegishvili et al., 2012).

Plant uptake offers another mechanism to bioaccumulate gold. Plants have been used for gold accumulation due to its ability to solubilize metals or minerals via root uptake from region surrounding the primary deposits of gold (Anderson et al., 1999). Specialized microbe inside/outside plant roots adapt and evolves their metabolism to solubilize the gold particles. Such plants have established certain responses (either genetic or biochemical) to deal with stress associated with foreign toxic gold complexes. Plant microbes are capable to mediate gold solubilization by promoting gold oxidation and excrete stabilizing ligands ions for gold which can form complex or colloids with gold. Accordingly, they precipitate those gold complexes intra- and extracellularly in form of nanoparticle and this ability of the microbes has been widely exploited for phytoremediation and phyto-mining (Wilson-Corral et al., 2012). Gold uptake efficiency of Arabidopsis thaliana L. (Arabidopsis) and its physiological and genetic responses towards gold has been tested in-vitro and revealed that gold uptake preferences of different parts of this plant differs for oxidized and reduced gold where former can be observed in both roots and shoots, but reduced gold can only be found in the roots (Taylor et al., 2014). This study proposed the necessity of an ionic form of gold for its uptake by plants, exposure of which stimulates the up regulation of certain genes associated with plant stress with simultaneous down-regulation of specific metal transporting genes to minimize gold uptake (as a response towards such stress). Understanding the unique plant-gold interaction by studying the molecular response of plants towards AuCl₄⁻ ions using transcriptome analysis can assist us to control the biosynthesis of AuNPs. Upregulation of various genes takes place in plants in response to gold toxicity, which includes oxidative stress, metal ion transport, metal ion/glutathione binding sites and revealed glutathione mediated metal detoxification followed by the biosynthesis of plant hormone. For instance, plants are known to mediate ABA-mediated signalling on getting exposed to toxic AuCl₄⁻ ion, which are often caused by excessive accumulation of responsive core elements for abscisic acid inside plants (Shukla et al., 2014). Biosynthesis of gold nanoparticles in Arabidopsis plant (roots/and shoots) has been studied (at transcriptome/proteome level) for its ability to biotransform KAuCl₄ ions, and an upregulation of few gene families (like WRKY, MYB, BHLH) has been observed in roots combined with glutathione s-transferases induction in shoot during AuNPs biosynthesis (Tiwari et al., 2016). The gold-sulphide mineralisation using Acidithiobacillus ferrooxidans (iron-/or sulfur-oxidizing microbes) helps in complexation of the gold by allowing it's solubilisation and re-concentration during the process (Reith et al., 2012). Plants like hemp are known to accumulate several heavy metals by their dissolution and gold can be harvested/recovered by its incineration, which however, can cause air-pollution, so calls for more sustainable alternatives to make process more clean and sustainable (Fashola et al., 2016). Formation of gold nanoparticles using aqueous plants like Coleus aromaticus, Elaeis guineensis, Cinnamomum camphora (leaves extract), Juglans regia (green husk extract), Annona squamosa (peel extract), Avena sativa (biomass) and Diospyros Kaki (fruit extract), which reportedly said to possess an excellent capability to turn Au(III) into Au(0) (Irfan et al., 2017; Ahmad et al., 2018; Boomi et al., 2019; Huo et al., 2019). Bioaccumulation inside the living systems enforces various limitations which are yet to be overcome, which includes cellular growth inhibition at high gold concentrations or at extreme pH conditions or high salt concentrations. The gold bioaccumulation potential of native microbes, however, has significantly enhanced by genetically modifying them to incorporate endurance towards excessive toxicity of gold along with operability at wider range of pH. Despite of economic competitiveness of biominning mechanisms, these microbe-assisted gold recovery methods has several limitations of its own. Excessive harshness of gold ore concentrates proves lethal to living cells. Also, more controlled bioreactor-based biominning of gold is required to achieve high, but stable yield during different batches along with high specificity to gold at wider ranges of pH and temperature.

3. Microbe-gold interactions mediated gold recovery, principle and mechanisms

Survival mechanism of a microbe influences its interactions with metal ions. Bacteria-metal interactions form the very foundation of technological innovations for the geochemical detection methods and real-time control of mineral processing systems for gold exploration. Previously, several studies has been conducted to decode gold-microbes interactions in supergene conditions by primarily using the aqueous solutions containing either AuCl₃ and AuCl₄⁻ ions (Nakajima, 2003) or Au(S₂O₃)²⁻ (Reith et al., 2007a). Direct and indirect participation by microbes (such as Bacillus subtilis) and gold nuggets formation has been validated by researchers and mechanism of which has widely investigated (Fig. 4, Shedbalkar et al., 2014; Salunke et al., 2016). Detailed account of microbe-gold interactions has also been reviewed previously (Savvaidis et al., 1998).

The charge (negative or positive) and ligand type of gold nanoparticle have direct role in inducing cellular toxicity and surface binding to the microbial cell surface. Correlation between the surface of gold nanoparticles with cell viability has been validated for microbes with different cellular surface properties i.e., using gram negative bacteria Sheuvenella oneidensis (with outer-/and cytoplasmic membranes and pep- tidoglycan layer) and gram-positive bacteria i.e., Bacillus subtilis (with thick peptidoglycan layer, teichoic acid and cytoplasmic membrane). Negative charges on both microbial surfaces displayed higher surface interactions with the cationic poly-electrolyte (poly-allylamine hydrochloride or 3-
mercaptopyrrole) than anionic one (3-mercapto-propionic acid) due to the interplay of electrostatic interactions that favours opposite charges, which causes severe gold toxicity and damages the bacterial cell wall that prove fatal to both bacteria’s (even without internalising any AuNP inside the cell) (Feng et al., 2015). Previously, mechanism of microbial mode to biosynthesis gold nanoparticles has been reviewed briefly (Shedalkar et al., 2014). In addition, different environmental zones possess different groups of microbes such as chemolithoautotrophic microbes like iron- and sulphur-oxidizing bacteria (found on soil surface or subsurface environment) also known to precipitate gold. These microbes form biofilms on the gold-enriched metal sulphides (i.e., pyrites and arsenopyrites) surface to harness metabolic energy for its growth by oxidizing them by using different pathways (like sulphur oxidase pathway and reverse dissimilatory sulphite reductase pathway). Biogeochemical cycling of gold by biofilms (either active or remnant) takes place by helping in Au dispersion (via Au nanoparticles formation) or and acts as the reaction centre of various microbial processes that lead to gold formation by stabilizing gold for microbial metabolism/direct uptake (Reith et al., 2010).

3.1. Geological microbial dynamics and gold

Earth soil is stratified into various environmental zones and each zone inhabited by different microbial types that assists in thiosulphate production that further promotes solubilization of the gold. In aerobic zones (soil surface/or subsurface), gold can be recovered by oxidation of gold sulphide minerals by atmospheric oxygen in presence of iron and sulphur oxidizers, which helps in gold complexation that from thiosulphate (Aylmore and Muir, 2001; Southam and Saunders, 2005). In addition, a soil actinomycete, Streptomyces fradiae can metabolize the sulphur from cystine and form thiosulphate;

\[
2\text{FeAsS} \rightarrow \text{Au} + 7\text{O}_2 + 2\text{H}_2\text{O} + \text{H}_2\text{SO}_4 \rightarrow \text{Fe}_2\text{SO}_4\text{SH} + 2\text{H}_2\text{AsO}_4 + \text{[Au]} \quad (8)
\]

Growing cyanogenic biofilms (Chromobacterium violaceum and Pseudomonas plecoglossicida) on gold containing e-waste has successfully solubilized 100% and 69% of gold within 10–17 and 3 days respectively (Campbell et al., 2001; Faramarzi and Brandl, 2006). Generally, HCN synthase (an enzyme complex attached to microbial membrane) helps cyanogen’s in synthesizing the cyanide (a typical secondary metabolite which makes microbial species tolerant towards and help withstand associated toxicity) in presence of a metabolic precursor called glycine (Castric, 1975; Knowles, 1976; Rodgers and Knowles, 1978; Wissing and Andersen, 1981; Faramarzi and Brandl, 2006).

3.3. Mechanism of gold biorecovery

Mechanism of elemental gold precipitation by \(\text{Au(S}_2\text{O}_3\text{)}^2\)\(^–\) degradation by Acidithiobacillus thiooxidans has been thoroughly studies, which can use it as an energy source in unavailability of other sources in auriferous aqueous solution and can precipitate gold intra-or extracellularly (inside cellular periplasm and on cytoplasmic membrane surface) in fine-grained colloidal forms suggesting entrance of \(\text{Au(S}_2\text{O}_3\text{)}^2\)\(^–\) inside cell as a complex form, which de-complexes itself as shown

\[
\text{Au(S}_2\text{O}_3\text{)}^2\rightarrow \text{Au}^+ + 2\text{S}_2\text{O}_3^2\quad (11)
\]

Although A. thiooxidans shows more affinity towards thiosulfate than \(\text{Au(S}_2\text{O}_3\text{)}^2\)\(^–\), which is being utilized only when none of other sources are available. Chemicals species like thiosulfate or tetrathionate enters inside microbial cell through pores at its cellular surface and get oxidized in its periplasm by undergoing a series of metabolic reactions (Fig. 5, Schippers and Sand, 1999). Gold transportation inside the microbe takes place either by chemiosmotic gradient or through adenosine triphosphate hydrolysis. Microbes tolerate such gold toxicity by reducing toxic Au\(^3+\) form to less toxic Au\(^0\) form, or combining it with something to yield a compound, or ejecting it out of its body (Nies and Silver, 1995). Interestingly,

\[
\text{Au} + 0.5\text{O}_2 + 2\text{H}^+ + 2\text{S}_2\text{O}_3^2\rightarrow \text{[Au(S}_2\text{O}_3\text{)}^2\text{]}^+ + 2\text{S}_2\text{O}_3^2+ + 2\text{H}_2\text{AsO}_4 + \text{[Au]} \quad (9)
\]
intracellular uptake of Au(S2O3)23−/C0 by microbes often proves lethal to the microbe due to its adverse effect on its electron transport chain. Extracellular gold formation in solution Au(S2O3)23−/C0 by microbes can be specifically produced only by live microbes than dead ones, however some believes organic acids being released by dead microbes triggers formation of gold (Fig. 6, Lengke and Southam, 2005).

4. Futuristic technologies for gold bio-recovery

4.1. Hybrid chemical-microbial gold biosorption

Over the past few years, microbes have been applied as a metal sink and being capable to bind with metals ions in and/or around cells or cell walls/or extracellular polysaccharides by precipitating them intra-or extracellularly. Many studies have recommended use of compatible combination of chemical with efficient microbial agents to recover higher level metal ions (Pant et al., 2014; Singh and Pant, 2016). Integrated leaching-sorption method for effective gold recovery combines negligibly toxic chemical like ammonium thiosulfate (AT) with microbe Lactobacillus acidophilus assisted biosorption to acquire the gold recovery rate of 90%. Microbial consortium constitutes a matrix on the solid metal ions of liquid auroferous suspension, which acts as a playground for microbes to perform alchemical reactions (such as acidolysis, complexolysis, and redoxolysis) to transform and uptake the gold by biosorption (Ledin and Pedersen, 1996). The AT improves the π−π interaction which strengthens amide absorption bonding between AT-LA. Few days of incubation with AT helps in improving the amide bond strength by enhancing its π−π interaction with bacteria, which fastens the gold biosorption from AT leachant. Combining various biological methods with moderate usage of non-hazardous chemicals has a promising scope in creating high-end hybrid technologies for the gold metallurgy, but more studies need to be carried out to make clear distinct conclusion about positive or negative effect of using integrated hybrid technologies.

4.2. Microbial electrochemical system assisted gold recovery

Microbial electrochemical systems (MES) is emerging as a promising technology for gold biorecovery especially by utilizing e-wastes. Encouragingly, gold recovery of 91% ± 1% can be accomplished by using planktonic bacteria based MEC. Interestingly, mode of extracellular electron transfer (EET) from/or to microbes to electrodes were mostly found to be indirect EET (Romero, 2018). High hydrogen evolution reaction in MES has be achieved with less concentrated Au(III) containing wastewater under acidic pH, for example, attaining Au recovery of 97.84% ± 0.03% at lower concentrations (50 mg/L), but only 88.37% ± 0.05% at 200 mg/L (pH 2) as electrons produced by microbes were not sufficient enough in the later to reduce the excess of Au at higher concentrations. Also, simultaneous occurrence of two competing reductive reactions, i.e., proton reduction reaction beside Au(III) reduction reactions contribute to extra charge to overall charge produced by the MEC (Romero, 2018).

In MES, Shewanella oneidensis has been applied as a metal-reducing microbe to intracellularly precipitate the Au nanoparticles by reducing Au(III) to Au(0) by nucleation and electrodeposition (De Corte et al., 2011). Also, mechanisms of Au(III) to Au(0) bio-reduction by S. oneidensis MR-1 has been deduced and effect of intracellular formation of AuNPs on physical and biochemical properties of microbes has also been investigated. Interestingly, wild and mutant strains of S. oneidensis reacts differently to initial exposure to Au(III)
Fig. 6 – Depiction of sulfur-bacterial (A. thiooxidans) responsiveness towards gold exposure (Au(S₂O₃)²⁻). Adapted and modified (Lengke and Southam, 2005).
containing wastewater, where performance of MES based on former get deteriorated over time due to intracellular AuNP deposition, however mutant strains (with no AuNP formation) based MES performance remains unaffected (Wu et al., 2013). Another strain, Shewanella halotolerans was used to attain electrochemical recovery of AuNPs from AuCl₄⁻ leachate (Zhu et al., 2016). However, fostering MES applicability in terms of electroactive microbe assisted gold recovery at large scale still requires vigorous research to gain an in-depth understanding of microbe-gold associated electro-kinetics and electron transfer mechanisms. Gold exploratory research based on the electrometallurgy has wide range of applicability and its high-end gold selectivity make it a promising technology of future. Major challenge of these methods is to choose economical soluble electrodes or mediators (as electron donor or acceptors) for gold recovery and to optimise their minimal concentrations for gold metal-recovery through MES platform. Although, the use of cheap solid electrodes (instead of using soluble electron donors/or acceptors) helps in making process highly cost-effective and specific, which can be control by varying the external electrode potential adding selectivity to metal bioaccumulation.

5. Conclusion and outlook

Global increased practising of biomining methods is a positive commencement of sustainable microbe mediated harvesting of precious metals from e-wastes and metal infested soil. Elucidating the defining role of microbes in biomining technologies clearly indicates its exceptional potential to bio-mine gold without causing any hazards. Gained understanding of microbe-gold interactions by studying the gold fixing mechanism in these gold harvesters will help future researchers to find novel loops in pathways which can be altered to enhance their gold harvesting potential. However, researches based on enhancing the metabolic mechanistic of gold harvesting microbes at molecular level (using high-throughput analytical technologies) are still in its infancy. Although, our knowledge about the role of changing microbial dynamics and interspecies crosstalk on gold solubilisation has been improved since past few years yet it is somewhat limited by our lack of understanding of microbial mechanism of gold solubilisation. This paper has discussed the biological aspects of microbe mediated gold-mining processes, with prime focus on depicting the recent advancements in gold biomining processes in terms of applied microbial strains and associated mechanisms.

The major challenge associated with gold mining is to recover pure gold from multi-metalled low-grade ores contained inside e-wastes or metal infested soils, which however, can be solved by incorporating the bio-electrometallurgical technologies with biomining methods to ensure highly specific retrieval of gold. Additionally, prospective researchers can further incorporate recent biotechnological methods with bio-electrochemical-biomining methods to maximise commercial suitability of biological methods which can process gold ore concentrates with high pulp density and alkalinity. Despite proven benefits of bioleaching, the process is still restrained by inadequacy of its gold retrieval efficiency, which is another challenge to conquer in order to make it an attractive gold mining alternative for industrial bio-miners globally. While the aftermaths associated with irrational use of cyanide producing microbes cannot be denied, careful and calculated optimisation of microbial strains at laboratory scale surely will help to avoid future aftermaths associated with intoxicating microbial usage. Additionally, reactor-based bioleaching has its own limitations such as inadequate aeration, need for prolonged residence time, continuous agitation to ensure proper mixing/aeration and dealing with low-grade ore and lower pulp densities which need to be overcome to make reactor scale bioleaching an affordable and economical option for the investors of low scale gold biomining. Biominalization of gold is hindered by inaccessible of microbes due to matrix encapsulation of gold which can be improved by implicating suitable biological pre-treatment methods which ensures maximal release of the gold ions, thereby improving the gold mineralisation by improving selective exposure of the gold ions to intra- or extracellular biogenic metabolites or gold mineralising enzymes of applied microbes. Varying gold adsorptive potential of the living and non-living microbes with changing gold mining sites indicates the dynamic responsiveness of microbes towards geographical location during biosorption, which can be an interesting area of research for researchers. Innovative sensitive biogeochemical detection methods and biosensors for gold-specific exploration will revolutionise the gold exploration by applying the basics of these bacterial-metal interactions, thereby making the real-time gold processing systems into a reality. Considering the long-term decline of ore grades, microbe-mediated biological concentration of gold seems the only sustainable refuge for clean recovery of precious metal like gold, though technological breakthroughs in precious metal bio-mining is just the beginning of new era of biomineral research.

Conflict of interest

None declared.

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References


Das, S., Ting, Y.-P., 2017. Improving gold (bio) leaching efficiency through pretreatment using hydrogen peroxide assisted sulfuric acid. CLEAN Soil Air Water 45, 1500945.


Jerez, C.A., 2017. Biomining of metals, how to access and exploit

Johnston, C.W., Wyatt, M.A., Li, X., Ibrahim, A., Shuster, J.,
Southam, G., et al., 2013. Gold biominalization by a
Biol. 9, 241.

bioreactor for recovering precious metals from waste printed

Kalabegishvili, T.L., Kirkesali, E.I., Rcheulishvili, A.N.,
Synthesis of gold nanoparticles by some strains of Arthrobacter

saccharide-mediated reduction of Au\(^+\) to gold nanoparticles,
new insights for heavy metals biominalization on microbial

Recent progress of algae and blue–green algae-assisted
synthesis of gold nanoparticles for various applications.

40, 652.

Knowles, C.J., Bunch, A.W., 1986. Microbial Cyanide Metabolism:
Advances in Microbial Physiology. Elsevier, pp. 73–111.

systems for the extraction and recovery of metals from

Kuyucak, N., Volesky, B., 1989b. The mechanism of gold
oxidizing bacteria on the stability of the gold-thiosulfate
complex. Geochimica et Cosmochi Acta 69, 3759

Ledi, M., Pedersen, K., 1996. The environmental impact of mine
wastes—roles of microorganisms and their significance in

Lengke, M.F., Ravel, B., Fleet, M.E., Wanger, G., Gordon, R.A.,
Southam, G., 2006. Mechanisms of gold bioaccumulation by
filamentous cyanobacteria from gold (III)– chloride complex.

oxidizing bacteria on the stability of the gold-thiosulfate

Li, G.-X., Zhou, S.-Y.-D., Ren, H.-Y., Xue, X.-M., Xu, Y.-Y., Bao, P.,
2018. Extracellular Biominalization of gold by Delftia
tsuruhatensis GX-3 Isolated from a heavy metal contaminated

Madarig-Arias, J.E., Argumedo-Delira, R., Alarcón, A., Mendoza-
Bioleaching of gold, copper and nickel from waste cellular
phone PCBs and computer goldfinger motherboards by two

Mata, Y., Torres, E., Blazquez, M., Ballester, A., González, F.,
Munoz, J., 2009. Gold (III) biosorption and bioreduction with
the brown alga Fucus vesiculosus. J. Hazard. Mat. 166, 612–618.

Maulani, N., Li, Q., Sand, W., Vera, M., Zhang, R., 2016.
Interactions of the extremely acidophilic archaean Ferroplasma
acidiphilum with acidophilic bacteria during pyrite bioleaching.

Mbayo, J.J.K., Simonsen, H., Ndlovu, S., 2019. Improving the gold
leaching process of refractory ores using the Jetleach reactor.

McGivney, E., Gao, Y., Liu, Y., Lowry, G.V., Casman, E.A.,
promotes dissolution of gold nanoparticles in soil. Environ.

Moore, B., Duncan, J., Burgess, J., 2008. Fungal bioaccumulation of
copper, nickel, gold and platinum. Miner. Eng. 21, 55–60.

Improving gold recovery from refractory gold ores through
biooxidation using iron-sulfur-oxidizing/sulfur-oxidizing

Munoz, J., Gonzalez, F., Blazquez, M., Ballester, A., 1995. A study of
the bioleaching of a Spanish uranium ore. Part I, A review of
the bacterial leaching in the treatment of uranium ores.
Hydrometallurgy 38, 39–57.


Nancharyaiah, Y., Mohan, S.V., Lens, P., 2016. Biological and
electrochemical recovery of critical and scarce metals.
Trends Biotechnol. 34, 137–155.

Natarajan, G., Ting, Y.-P., 2015. Gold biorecovery from e-waste,
an improved strategy through spent medium leaching with P

Natarajan, G., Ting, Y.P., 2013. Two-step bioleaching and spent
medium leaching of gold from electronic scrap material using
Chromobacterium violaeum. In: Advanced materials research.


Geological Survey.

Pant, D., Joshi, D., Upreti, M.K., Kotnala, R.K., 2012. Chemical and
biological extraction of metals present in E waste: A hybrid
technology. Waste Manag. 32, 979–980.

Pant, D., Singh, P., Upreti, M.K., 2014. Metal leaching from cathode
ray tube waste using combination of Seratia plymuthica and
EDTA. Hydrometallurgy 146, 89–95.

Parial, D., Gopal, P.K., Paul, S., Pal, R., 2016. Gold (III) bioreduction
by cyanobacteria with special reference to in vitro biosafety

Park, S.-I., Kwak, I.S., Bae, M.A., Mao, J., Won, S.W., Chung, Y.S.,
et al., 2012. Recovery of gold as a type of porous fiber by using
biosorption followed by incineration. Biore. Technol. 104,
208–214.

Progressive biogeochemical transformation of placer gold
particles drives compositional changes in associated biofilm

Rea, M.A., Zammit, C.M., Reith, F., 2016. Bacterial biofilms on gold
grains—Implications for geomicrobial transformations of

Reith, F., Etschmann, B., Grosse, C., Moors, H., Benotmame, M.A.,
biominalization in the bacterium Cupriavidus metallidurans.

Reith, F., Fairbrother, L., Nolze, G., Wilhelmi, O., Clode, P.L.,
Gregg, A., et al., 2010. Nanoparticle factories, biofilms hold the
key to gold dispersion and nugget formation. Geology 38,
843–846.

The geomicrobiology of gold. ISME J. 1, 567.

Reith, F., McPhail, D., 2006. Effect of resident microbeota on the
solubilization of gold in soil from the Tomakin Park gold mine,
New South Wales, Australia. Geochim. Cosmochim. Acta 70,
1421–1438.

Reith, F., McPhail, D., 2007. Mobility and microbially mediated
mobilization of gold and arsenic in soils from two gold mines
Acta 71, 1183–1198.

Biominalization of gold, biofilms on bacterioform gold.
Science 313, 233–236.


