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Immobilized reaction system for the Bio-oxidation of Gold sulphide

Sujata Dabolkar[1] and Nandkumar M. Kamat[2]*

Mycological Laboratory, Department of Botany, Goa University, Taleigao, Goa 403 206, India

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ABSTRACT

Bio-oxidation refers to a pre-treatment process that uses bacteria to catalyse the degradation of mineral sulphides, usually pyrite or arsenopyrite, which host Gold, silver or both. Biomining through bioleaching and bio-oxidation recovers desired metals at the required specifications with lower environmental impact and costs from ores and waste streams. During this study Novel Sulphur Oxidizing Bacteria (NOSOB) was isolated from the Banded Iron Formation of Goa and this was used for the bio-oxidation of separated Gold sulphide from the same samples. The experiment was set up in a Immobilized reaction system for the Bio-oxidation of Gold sulphide.

Key words: *Immobilized, Bio-oxidation, Gold, Gold sulphide, Reaction system, NOSOB*

Introduction

Metal sulphides

Small particles of Gold, a metal of higher interest, are mostly found in a matrix of sulphide-based minerals. In the Gold mining industry, the precious metal is generally found in a sulphide mineral matrix (Podder *et al.,* 2022). Certain acidophilic bacteria have the ability to aid the oxidative dissolution of sulphide minerals. This is very promising in extraction of precious metals within assisted microbial activity (Johnson, 2008). During these processes insoluble metal sulphides are oxidized to soluble metal sulfates. The majority of the world's Gold is associated with sulphide mineral deposits located at ancient convergent plate boundaries in areas such as South Africa, Canada, California, Brazil, southern India, Russia, and Western Australia (Monroe *et al.,* 2007). The particle sizes of sulphide minerals and their concentrations have significant impact on Gold dissolution (Bas *et al.,* 2018). High sulphide containing ores should be given adequate pretreatment to fully or partially break down the sulphide matrices to liberate Gold, followed by Gold leaching under oxygen atmosphere (Saim *et al.,* 2022).

Metal sulphide Bio-oxidation

Bio-oxidation has been increasingly used in heap, dump and in situ leaching, bacterial oxidation of sulphide minerals and has also acquired industrial significance in recent years). Biomining through bioleaching and bio-oxidation recovers desired metals at the required specifications with lower environmental impact and costs from ores and waste streams (Darvanjooghi *et al.,* 2022). The involvement of metals in microorganisms growth, metabolism, and differentiation are interdependent to the metal species and microbial environment as well (Ilyas *et al.,* 2022). Several studies have shown that Gold solubilization occurs when microorganisms such as *Chromobacterium violaceum* which directly attach to

ORCID ID: https://orcid.org/0000-0001-6141-3069
ORCID ID: https://orcid.org/0000-0003-1070-049

the surface of Gold, forming biofilms and cyanide (Campbell *et al.*, 2001; 2018). *Acidithiobacillus ferroooxidans* has been used in the oxidation of elemental sulphur to the reduction of ferric iron in the goethite fraction of a limonitic nickel ore at 30°C, metals such as Ni, Co, Cr and Mn were effectively solubilized (Johnson *et al.*, 2014). Studies on auriferous soils from Australian mine sites have shown that the resident microbiota are capable of solubilizing up to 80 wt. of the Au contained in these soils (Reith *et al.*, 2006). Bio-oxidation of Gold-, silver, and antimony-bearing highly refractory polymetallic sulphide concentrates, and its comparison with abiotic pretreatment techniques has been reported (Tanaka *et al.*, 2015).

Microbes involved in Bio-oxidation

A variety of mineral oxidizing bacteria serves as a biological tool and can easily oxidize iron and sulfur containing minerals. These include the iron- and sulfur oxidizing *Acidithiobacillus ferrooxidans*, the sulfur-oxidizing *Acidithiobacillus thiooxidans* and *Acidithiobacillus caldus* and the iron-oxidizing *Leptospirillum ferrooxidans* and *Leptospirillum ferriphilum* (Clark and Norris, 1996; Nagpal *et al.* 1993; Siddiqui *et al.*, 2009). Bacteria such as *Proteobacteria, Actinobacteria*, and *Firmicutes* are involved in oxidization and also to dissolve metal sulphide minerals, such as ferrous iron (Fe_2S) and sulfur compounds (Garrido-Cardenas *et al.*, 2020; Johnson, 2014; Pakostova *et al.*, 2020). There are reports on extracellular polymeric substances overproduction strategy in *Ferroplasma acidiphilum* growth for bio-oxidation of low-grade Gold bearing ore (Darvanjooghi *et al.*, 2023).

Methodology

Sample preparation

Collected samples from mining belts of Goa (Dabolkar and Kamat., 2022) were dried, powdered using mortal and pestle (Panda *et al.*, 2011). The recovered samples were concentrated by panning method (Southam *et al.*, 2009) and subjected to magnetic separation using neodymium magnet (Oliveira and Larizzatti, 2006) and sieved into different fractions with the sieve size of 250, 150, 106, 53 μm. The details of the samples and procedure of obtaining Gold sulphide is given in the previous publication (Dabolkar and Kamat, 2022).

Immobilized reaction system

Source of culture

Highly sulphide oxidizing strain isolated from mining belts of Goa and designated as NOSOB was cultivated aerobically at 28–30°C and 150 rpm in an Erlenmeyer flask with 50 ml of a specific media (9K) without sulphur source (Garcia, 1991). The pH was adjusted at 2 with concentrated H_2SO_4. For the bench top experiment, inoculums of ca. 10^8 cells/ml were transferred to flasks with 100 ml of culture media, pH 2 without a source of sulphur (García-Meza *et al.*, 2012). NOSOB was identified by 16S sequencing at Muppandal Genomics and Immunological private limited, Hyderabad, Telangana.

Experimental design

The bio-oxidation experiment was carried out in a 1000 ml Borosil beaker (BRL_1000D29). The surface sterilized thermocol with absolute ethanol was placed at the base of the beaker. The slides (75 mm x 25 mm HIMEDIA were coated in a coverslip size area with 1.5 ml of sterile 9 K medium mixed with culture. The volume of sterile 9K medium used to immobilize culture (X) was 40 ml and that of culture (Y) was 10 ml. The crude Gold sulphide used in this experiment was obtained by previously described technique (Dabolkar and Kamat, 2022). About four grams of Gold sulfide was immobilized at the centre of 21 slides in 400 mm^2 area and distributed evenly to get a concentration of 190 mg/slide. Total 10 slides were placed in slits made in the thermocol and placed in the beaker well separated from each other. The beakers were filled with 600 ml of dilute HCl with pH 2 so that coated area of the slide is completely dipped in the acid. Oxygen was bubbled through under pressure continuously using an external aerator. The beakers were completely covered with the aluminium foil and kept at 32 degrees Celsius. The design is schematically represented in the Figure 1.

Monitoring of the slides for formation of Gold

After the incubation, the slides were collected at a regular interval of 3 days for 30 days to measure the efficiency of bio-oxidation and Gold recovery (Campbell *et al.*, 2001). After every 3 days each slide was studied under microscope. The edges of the slides showing positive Gold deposition results were cut. Heavy Gold particles were retained. This metallic Gold fraction was mounted on a slide with

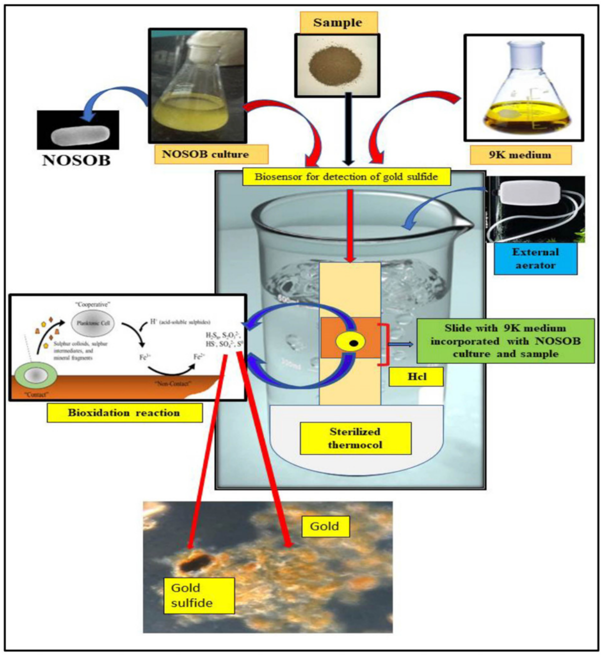

Fig. 1. Schematic representation of experimental design for Bio-oxidation by immobilized reaction system.

DPX after drying and checked for Gold. Slides were also prepared with few drops of the Aqua regia to check formation of metallic shining film. Control slides were treated in the same manner.

ICP-AES studies

Replace with following:

One gram of the sample of immobilized agar from Gold positive slide was collected from the slide and dissolved in 5 ml of aqua regia. Further it was boiled to digest, diluted with water and sent for ICP-AES (Model: Perkin Elmer, U.S.A.; Make: Avio 200) analysis at Sophisticated Instrumentation Centre for Applied Research and Testing (SICART) supported by Department of Science & Technology (Govt.of India) managed by Charutar Vidya Mandal (Liang-Cheng *et al.,* 2018).

Results

Immobilized reaction system

Bio-oxidation experiment was carried out successfully in a 1000 ml Borosil beaker (BRL_1000D29) with the help of slide-based coating technique. We were successful in standardizing the concentration of medium and culture so that the medium gets solidified and pH is maintained. Set up of immobilized system for bio-oxidation, placement of the slides inside the beaker and external aeration as seen from the top is seen in Figure 2.

Monitoring of the slides and formation of Gold

There was formation of colloidal Gold forms and detection of flux lines of colloidal Gold on on all test slides. Evidence for formation of sulphuric acid as product of biooxidation was seen. The formation of flux of sulphuric acid depolymerizes the agar and creates rough surface. In two-dimensional slide coating it appears that the oxygen gradient is on five sides, the four edges and the surface. Therefore, Oxygen diffusion was found to be highest from the edges and declines towards the centre. Oxygen diffusion from surface was not uniform depending on thickness of the layer. Therefore, the reaction is rapid at the edges and shows clearance of sulphide, formation of complex biofilms and flux of sulphuric acid which depolymerizes the agar and creates rough landscape. The Gold formed after oxidation is typically microbioform. After 14 days the process of Gold extraction was completed. It was found the pH

Table 1. Microorganisms involved in Au biomining (Source: Compiled from Brombacher *et al.,* 1997; Rea *et al.,* 2016, 2018; Reith *et al.,* 2009; Rizki *et al.,* 2018; Reith and Shuster, 2018; Shuster *et al.,* 2018).

Micro-organisms	Microbial processes
Acidothiobacillus ferroxidans	Fe oxidation
Acidothiobacillus thiooxidans	S-oxidation
Fe- and S-oxidizer (*A. ferroxidans, Streptomycetes fradiae*)	Thiosulphate production
Cyanide producing bacteria (*Pseudomonas* spp, *Chromobacterium violaceum*)	Cyanide production
Bacteria such as *Corynebacterium glutamicum*, Fungi such as *Penicillium* spp	Amino acid production
Fungi such as *Fusarium oxisporum*,	Bio-reduction of Au absorption
Cupriavidus metallidurans	Precipitating nanometre-scale platinum particles
Acidocella aromatica	Precipitation and bioreduction

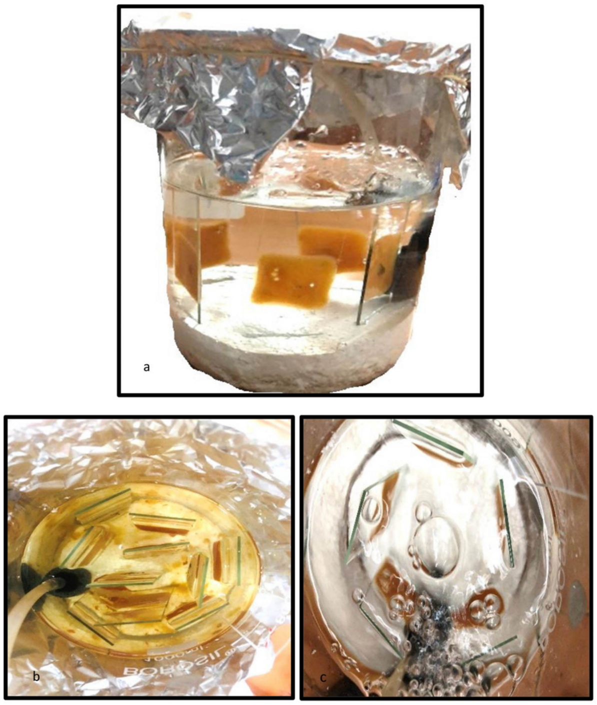

Fig. 2(a-c). Set up of immobilized system for biooxidation; b- Placement of the slides inside the beaker; c-external aeration as seen from the top

was dropping from 2 and it reached 1.8 by a week and later 1.5 by 14 days. Gold formed at all the edges of the slide can be seen in Figure 3. There was the formation of colloidal Gold forms and the detection of flux lines of colloidal Gold on the slides. The concentration of Gold reported was 2133.5 mg/L.

Discussion

The detection and separation of Gold sulphide in the heavy fraction of Banded Hematite Quartzite (BHQ) sourced from the BIF in Goa has been reported

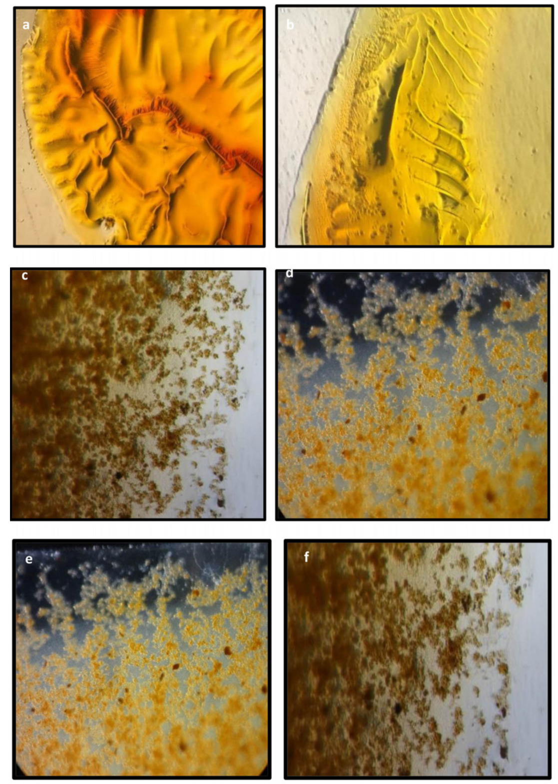

Fig. 3(a-f). a,b-Film of Gold formed after dissolving in aquaregia indicating the formation of Gold; (c-f)- Gold formed at all the edges of the slide

(Dabolkar and Kamat, 2022). Similar findings have been reported in Brazilian (de Oliveira and Larizzatti, 2005) and Australian mines (Reith *et al.*, 2006). There are reports on the bio-oxidation of Gold-, Silver, and Antimony bearing highly refractory Polymetallic Sulphide Concentrates, and its comparison with pretreatment techniques (Tanaka *et al.*, 2015). Several species of microorganisms have been identified for their potential application in bioleaching process. These can be bacteria, archaea, fungi and some species of yeast (Rezza *et al.*, 2001;

Table 2. Microorganisms potentially useful in Au Biomining

Microorganisms	Role in biosphere
Arthrobacter spp., *Corynebacterium* sp., *Deinococcus* sp., *Kocuria* sp., *Microbacterium* sp., *Micrococcus* spp.	1. Mediate initial colonization on the grains 2. Produce EPS 3. Conditions the grain surfaces
Pseudomonas spp., *Burkholderia* spp., *Methylobacterium* sp. *Acinetobacter* sp.	Production of EPS to further stabilize the biofilm
S-oxidizing or cyanide producing bacteria, e.g., *Diaphorobacter* sp., *Sphingomonas* spp.	Au solubilization
C. metallidurans, *Achromobacter* spp. and *Herbaspirillum* sp.	Metal-resistant bacteria with the ability to detoxify Au-complexes.

Kucera *et al.*, 2020). About 13 genera namely *Acidithiobacillus, Leptospirillum, Acidianus, Ferromicrobium, Sulfobacillus, Sulfolobus, Sulfurisphaera, Thermoplasma* and *Thiobacillus* found their applications in biohydrometallurgy (Liu *et al.*, 2016 and related references therein). Specifically, for bioleaching of sulphide minerals, autotrophic (acidophilic) bacteria have been used; whereas in the case of non-sulphide ores and minerals, heterotrophic bacteria and fungi have been used (Sajjad *et al.,* 2019). In these studies we have used (Novel sulphur oxidising bacteria) NOSOB culture which was isolated from Banded Iron formation (BIF) of Goa by Winogradskys technique (Dabolkar and Kamat, 2021). NOSOB was found to be gram negative, rod shaped, highly aerobic, motile, powerful sulphide oxidizer, growing at the pH of 1.8. experiment for extracting of Gold from local Iron ore sample and mining rejects. Chemolithotrophic microorganisms such as *Acidithiobacillus ferrooxidans*, gram-negative bacterium obtains energy by the oxidation of ferrous ions to ferric ions and uses molecular oxygen as the terminal electron acceptor in aerobic conditions. Therefore, the dissolved oxygen (DO) plays an important role in the bio-oxidation process of refractory Gold gram-negative bacterium which obtains energy by the oxidation of ferrous ions to ferric ions and uses molecular oxygen as the terminal electron acceptor in aerobic conditions. Therefore, the dissolved oxygen (DO) plays an important role in the bio-oxidation process of refractory Gold ore (Sun *et al.*, 2012; Wang *et al.*, 2015; Darvanjooghi *et al.*, 2022; Saim *et al.*, 2022). The formation of flux of sulphuric acid depolymerizes the agar and creates rough surface. There was drop in pH from 2 to 1.4 indicating formation of sulphuric acid (Deng and Liao, 2002; Climo *et al.*, 2000; Deng *et al.*, 2001). Previous studies have shown that *C. violaceum g*rown in nutrient broth formed a biofilm and could complex and solubilize 100% of the Gold on glass test slides within 4–7 days (Campbell *et al.,* 2002). The Gold formed after oxidation is typically microbioform. There was the formation of colloidal Gold forms and the detection of flux lines of colloidal Gold on the slide. The concentration of Gold reported was ICP AES results showed 2133.5 mg/l. Although the experiments were conducted on small-scales and further scaling-up will be needed to provide immediately implementable data.

The results by ICP-AES method indicated recovery of about 80% (w/w) Gold stoichiometrically from crude Gold sulphide. Gold recovery from this higher yield is very promising although. The cultures used in this experiment is possibly a novel taxa and further work is being undertaken to establish its specific identity. However, NOSOB in pure culture has shown the vast potential for isolation of similar efficient sulfide oxidizing bacteria from BIF. Our previous metagenomic work (Dabolkar and Kamat., 2023) has been found very exciting and promising in this direction. We claim that one simple immobilized system is easy to use under laboratory setup and can help future researchers working on Gold Biomining. It has not escaped our notice that substantial deposits of Gold sulfide available in rest of India and the world can be separated and subjected to action of cultures like NOSOB at appropriate scales to obtain vast quantities of metallic Gold. Our work is a humble step to add a new dimension to Gold mining industry.

Conclusion

For the first time in the world we have successfully demonstrated a microfluidics test, simple, benchtop, laboratory scale, easy to use technique incorporating a pure, viable, novel culture of sulphur oxidizing bacteria in standard basal medium to produce the required bio-oxidation reaction with crude Gold sulfide leading to recovery of metallic Gold with 80% (w/w) stoichiometric efficiency. Microscopic and ICP-AES technique confirms the success of the reaction design. This technique can be successfully used with both pure and crude Gold sulfide. Further work to modify and improve this technique has been undertaken.

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Conflicts of Interest

There is no conflict of Interest

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