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# Microbial aspects of acid mine drainage and its bioremediation

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**Abstract:** The role of chemolithotrophs such as *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* which were isolated from some abandoned mines and processed waste tailings in the generation of acid mine drainage and toxic metal dissolution was discussed. Mechanisms of acid formation and dissolution of copper, zinc, iron and arsenic from copper, lead-zinc and arsenopyrite-bearing sulfide ores and tailings were established in the presence of *Acidithiobacillus* group of bacteria. Sulphate Reducing Bacteria(SRB) isolated from the above mine sites could be used to precipitate dissolved metals such as copper, zinc, iron and arsenic. Arsenic bioremediation was demonstrated through the use of native microorganisms such *Thiomonas* spp. which could oxidize arsenite to arsenate. Bioremoval of arsenic through the use of jarosite precipitates generated by *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* was also found to be very effective. Biotechnological processes hold great promise in the remediation of acid mine drainage and efficient removal of toxic metal ions such as copper, zinc and arsenic.

**Key words:** acid mine drainage; acidithiobacillus; arsenic bioremediation; sulphate reducing bacteria; sulfide precipitation

## 1 Introduction

In India, as elsewhere in the world over, metal mining has been dubbed ecologically and environmentally unacceptable. Public complaints are often heard about mining activities causing pollution, forest degradation and displacement. In India mineral rich states include Orissa, Chattisgarh, Rajasthan, Jharkhand, Madhya Pradesh, Andhra Pradesh and Karnataka where extensive mining for copper, lead-zinc, aluminum, iron ores, coal, uranium and other industrial minerals are going on since centuries. Even though, mining produces mineral wealth, unscientific exploitation of earth's resources degrade land, water and forest cover. Water sheds, human habitation, surface and ground waters could be seriously contaminated and polluted over extensive regions. It has been estimated that over $2\times10^9$ t of environmentally-hazardous mined and processed wastes could be generated per year due to mining activities in India.

Mining is not environmentally benign and concerned industries need to consider and plan in advance for their present and future requirements for environmental control. There are several areas of environmental concerns in Indian mining such as cyanide discharges, acid mine drainage, disposal of metal-containing wastes and organic-ladden effluents. Abandoned mines and tailing dams containing millions of tonnes of sulfide mineral containing wastes pose a great threat to water tables and surface water streams in the vicinity. Major acid-producing mining operations are essentially confined to sulfide-bearing metalliferrous ores such as those of copper, lead-zinc, uranium, gold, coal, nickel, and cobalt. Pyrite ($FeS_2$), chalcopyrite ($CuFes_2$), pyrrhotite(FeS), arsenopyrite(FeAsS), galena (PbS), sphalerite(ZnS), pentlantite(FeNiS) and cobaltite (CoS) are a few hazardous sulfide minerals present in many of the nonferrous ore deposits and tailing dumps. Metal-ladden acidic solutions are generated when such sulfide mineral containing mined wastes are exposed to atmospheric conditions. Thousands of kilometers of streams are seriously contaminated by acid mine drainage in USA and Canada due to years of metal-mining. The role of native microorganisms in the generation of acid from such abandoned mines and processed wastes (tailings and over burden) is now well established.

The sulfur-bacteria cycle in nature is relevant with respect to biogenesis of sulfide-mineral deposits as well

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as generation of acidic effluents due to bacterial sulfide oxidation. For example, sulfide minerals present under the earth's crust such as pyrite ($FeS_2$), chalcopyrite (CuS·FeS), and pyrrhotite (FeS) are oxidized to their sulfates ($FeSO_4$, $CuSO_4$ etc) producing metal dissolved sulfuric acid solutions. Bacteria such as *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* ubiquitously present in sulfide mineral bearing ore deposits, mine tailings and abandoned mines bring about such acid producing biochemical reactions under natural conditions. These acidophilic autotrophic bacteria utilize ferrous ion and sulfur compounds as their energy source and reproduce through binary fission up to $10^8$ cells/mL in water. Consequently, *Acidithiobacillus* group of bacteria are responsible for the dissolution and mobilization of toxic metals such as copper, iron, zinc, cadmium, arsenic and nickel in acidic solutions generated from abandoned mines, mine wastes and tailing dumps[1].

In this respect, closed and abandoned mines are more prone to bacterially mediated acid generation and subsequent contamination of water tables and surface water streams.

In the author's laboratory, extensive investigations have been carried out to isolate acid producing bacteria from various Indian abandoned mines and tailing dumps. It is possible to establish the acid production potential of abandoned mines, mine overburden, waste soils and processed waste dumps through standard laboratory tests. Based on these tests, it becomes possible to predict acid production potential of active and abandoned mines as well as processed wastes. Remedial measures to mitigate acid water generation can then be undertaken.

## 2 Prediction of acid mine drainage

Identification of acid generation mines and predictions of successes and failures in mine waste management need be understood. The following aspects need be established before remedial measures for acid mine drainage are undertaken[2−3]. 1) Acid generation potentials and neutralization potentials of different ores and mineral wastes; 2) Potential toxic metal contaminants in the exposed rock minerals; and 3) Conditions favorable for exposure and transport of contaminants from mine sites.

Prevention is the best strategy and prevention holds the key in mitigating acid mine drainage.

Acid mine drainage(AMD) results from outflow of acidic water from abandoned mines and mineral wastes and occurs naturally within environments containing an abundance of sulfide minerals and microbial activity. When a mine is abandoned, the pumping ceases, and water floods the mine. Introduction of such water is the initial step in most acid rock drainage occurences. Tailings piles or ponds are also a significant source of acid rock drainage[4−6].

After being exposed to air and water, bcterial oxidation of sulfide minerals (often pyrite) within the surrounding rock and overburden generates acidity. Colonies of bacteria and archaea greatly accelerate the oxidation of sulfides. In particular, *Acidithiobacillus ferrooxidans* is a key contributor to pyrite oxidation:

$$2FeS_2+7O_2+2H_2O \rightarrow 2Fe^{2+}+4SO_4^{2-}+4H^+ \quad (1)$$

$$4Fe^{2+}+O_2+4H^+ \rightarrow 4Fe^{3+}+2H_2O \quad (2)$$

$$FeS_2+14Fe^{3+}+8H_2O \rightarrow 15Fe^{2+}+2SO_4^{2-}+16H^+ \quad (3)$$

The following are the major sources for acid mine drainage: 1) waste rock and tailings, 2) underground and open-cast mines, 3) stock piles, spoil piles, and 4) spent heap leach dumps.

Chemical quality of ARD can vary and is dictated by physical, chemical, mineralogical and microbiological properties of each site.

The primary requirements for acid generation are: 1) sulfide minerals in the overburden, 2) water content or a humid atmosphere, 3) presence of oxidant (usually oxygen) (gas phase and water), 4) pH levels, 5) temperature, 6) chemical activity of ferric ions, 7) surface area of exposed sulfide minerals, 8) chemical activation energy required to initiate acid generation, and 9) biological activity (such as iron and sulfur oxidizing bacteria).

The most commonly encountered bacteria responsible for acid generation through sulfide mineral oxidation are: 1) *Acidithiobacillus ferrooxidans* (an iron and sulfur oxidizer), 2) *Leptospirillum ferrooxidans* (an iron oxidizer), 3) *Acidithiobacillus thiooxidans* (is a sulfur oxidizer and cannot oxidize pyrite alone but grows on sulfur released after the iron has been oxidized), 4) *Thiobacillus thioparus* (oxidizes sulfides previously produced by SRB and is a neutrophile), and 5) Sulphate Reducing Bacteria (implicated in remediation through precipitation of metal sulfides).

Predicting Acid Drainage has been standardized through laboratory tests. The following questions can be answered through such tests. 1) What is acid generation potential and neutralization potential of different rock types exposed? 2) What potential contaminants/metals occur in the rocks that will be exposed? And 3) Under what conditions will transport of contaminants occur?

Most common acid producers are pyrite, pyrrhotite, bornite, sphalerite, galena, arsenopyrite and cobaltite. These sulfide minerals are associated with abandoned mines, waste rock piles and tailing dumps.

Static and kinetic tests are used in predicting acid mine drainage. Static testing is first step in understanding AMD potential of a mine. A prior

knowledge of various characteristics of rock types are essential to establish those components that are likely to generate acid and those which will neutralize acid. Acid base accounting(ABA) measures bulk amounts of acid generating and neutralizing materials.

Kinetic testing is more sophisticated and is the next step after ABA. Rate of acid generation in mine wastes in the presence of air, water and bacteria can be established. These tests are however more expensive and time consuming. However, it can provide data on rate of acidification over longer periods of time (months).

## 3 Experimental

### 3.1 Bacterial isolation, enumeration and characterization

The ore, tailings and water samples used for bacterial isolation were collected from different locations of copper, lead-zinc and gold mine sites in India aseptically in appropriate sterilized containers. All the samples were examined microscopically for the presence of bacteria. The physical appearance and physicochemical characteristics of the mine samples such as color, pH, and redox potentials were determined. Suitable media were used for the isolation of bacteria. A 5.0% (w/v) suspension of the ore sample was prepared by suspending 5 g of the ore sample in 100 mL of appropriate sterilized media in 250 mL Erlenmeyer flasks for bacterial isolation. The flasks were then incubated at 30 ℃ on an Orbitek rotary shaker maintained at 200 r/min. 9K-YE medium containing 0.05% (w/v) yeast extract and 25% (v/v) basal salt[7] was used for the isolation of *Thiomonas* spp. *Bacillus* spp. was isolated in Nutrient broth maintained at pH 7. Growth was monitored spectrophotometrically ($OD_{600}$). 9K medium was used for the isolation and growth of *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*[8]. Basal salts medium was used for growth of *Acidithiobacillus thiooxidans.* During bacterial growth pH, cell count, redox potential, ferrous and ferric iron concentrations were monitored. The culture flasks were periodically subcultured to obtain pure and actively growing cultures. Cell concentration was determined with a Petroff-Hausser counting chamber under a Leitz phase contrast microscope (Laborlux K Wild MPS12). Change in ferrous and ferric ion concentrations during the growth of *Acidithiobacillus ferrooxidans* was monitored by O-phenanthroline method using UV-260 Shimadzu UV-Visible Spectrophotometer. The isolated bacteria were observed after Gram staining. Biochemical tests were performed for the identification of the isolates. Similar isolation procedure was followed for isolation of Sulphate Reducing Prokaryotes using Postgate medium [9].

### 3.2 Agitation leaching and column leaching

Biodissolution of various ore and tailing samples was assessed in the presence and absence of *A. ferrooxidans* in 9K medium (no ferrous ions) at natural pH. Shake flask leaching studies were carried out in the presence of various ore and tailing samples.

In the case of shake flask leaching studies, 5 g of the ore/tailings sample was pulped to 100 mL using distilled water in 250 mL Erlenmeyer flask. The suspension was agitated on a rotary shaker at 200 r/min at room temperature. In the control flasks 10% alcoholic thymol solution was added as a bactericide and the pH was maintained at about 7. Parameters monitored included pH, bacterial cell number and metal concentrations. The shake flask experiments were carried out for a period of 300 d.

Pyrex glass columns were designed with 300 mm in height and 50 mm in diameter. The columns were packed with the ore/tailing samples on glass wool. These types of columns were set up in order to simulate conditions prevailing in actual tailing dump sites. Column leaching experiments were performed for a period of 300 d.

Pure mineral samples of pyrite, chalcopyrite and arsenopyrite were used to understand dissolution of arsenopyrite when present alone or in presence of pyrite and/or chalcopyrite in the presence and absence of *A. ferrooxidans*. 5 g of mineral samples were pulped in 100 mL of 9K medium for this purpose and agitated on a rotary shaker at 200 r/min at room temperature.

Dissolved copper, zinc, iron and arsenic concentrations were determined using ICP spectrophotometer.

### 3.3 Biological metal removal

Oxidation of the more toxic form of arsenic, namely, arsenite to arsenate in the presence of *Thiomonas* spp. and *Bacillus* spp. was established. Adsorption of arsenic (Ⅲ) onto the jarosites generated during the growth of *Acidithiobacillus ferrooxidans* was also determined.

Actively growing cultures of *Thiomonas* spp. and *Bacillus* spp. were inoculated into their respective media with the arsenite concentration being maintained at a final concentration of 30 mg/L using AR grade sodium arsenite. The flasks were then incubated on a rotary shaker at 30 ℃ maintained at 200 r/min. Residual arsenic (Ⅲ) concentration in the flasks was monitored as a function of time to establish its oxidation to As(Ⅴ).

The growth of *A. ferrooxidans*/*L. ferrooxidans* in 9K medium at pH 2 was continued for 72 h to generate sufficient amount of jarosite precipitate. After complete growth, the culture was filtered to separate the precipitate and its wet mass determined. To understand the effect of

pH on the adsorption of the arsenite species, studies were also carried out over a pH range varying from 2 to 9.5. Arsenic(Ⅲ) concentration was maintained at 10mg/L using sodium arsenite.

Sulfide precipitation of copper, zinc, iron and arsenic in the presence of isolated SRB was also established.

## 4 Results and discussion

Microbial habitat of some Indian abandoned mines and tailings dumps were determined in this work. Major objectives are given as 1) Isolation, culturing, characterization of chemolithotrophs implicated in Acid Mine drainage and use of Sulphate Reducing Bacteria (SRB) in remediation; 2) Role of microorganisms in Acid Mine Drainage and its remediation; and 3) Prevention and remediation strategies.

Dissolution and speciation of metals such as copper, zinc, iron and arsenic were assessed from ore and tailing samples collected from various mine sites. Acid Mine Drainage in the presence of *Acidithiobacillus* group of bacteria was established.

### 4.1 Isolation and identification of mining micro-organisms

Isolation of arsenic-specific microorganisms holds the key in understanding the microbiological aspects of arsenic dissolution, speciation and remediation.

Role of *Acidithiobacillus* group of microorganisms in the biodissolution of copper, zinc, iron and arsenic from various ores, tailings and mine wastes was established. Details of mines, samples received and bacteria isolated are illustrated in Table 1.

Morphological features of some isolated micro-organisms are given in Fig.1.

**Table 1** Microorganisms isolated from various Indian Mines

| Name of mine | Material | Bacterial isolated |
|---|---|---|
| Old copper mines sulfidic gold/silver mines | Copper tailings | *Acidithiobacillus ferrooxidans*<br>*Acidithiobacillus thiooxidans*<br>*Desulfotomaculum nigrificans* |
| | Sulfidic gold ore | *Acidithiobacillus ferrooxidans*<br>*Acidithiobacillus thiooxidans*<br>*Desulfotomaculum nigrificans* |
| | Acid mine water | *Acidithiobacillus ferrooxidans*<br>*Acidithiobacillus thiooxidans*<br>*Thiomonas* spp. |
| Open pit copper (chalcopyrite-pyrite) mines | Mine pit water | *Acidithiobacillus thiooxidans*<br>*Desulfotomaculum nigrificans* |
| | Mined ore | *Acidithiobacillus thiooxidans*<br>*Leptospirillum ferrooxidans* |
| | Old tailings | *Desulfotomaculum nigrificans* |
| | Fresh trailings | *Acidithiobacillus thiooxidans*<br>*Leptospirillum ferrooxidans* |
| Lead-zinc mines 1 | Near tailing dam | *Acidithiobacillus ferrooxidans*<br>*Desulfotomaculum nigrificans* |
| | Waste rock | *Acidithiobacillus ferrooxidans* |
| | Old tailings | *Acidithiobacillus ferrooxidans* |
| | Ore | *Acidithiobacillus ferrooxidans* |
| Lead-zinc mines 2 | Tailings | *Acidithiobacillus ferrooxidans*<br>*Leptospirillum ferrooxidans*<br>*Desulfotomaculum nigrificans* |
| | Ore | *Acidithiobacillus ferrooxidans*<br>*Leptospirillum ferrooxidans*<br>*Acidithiobacillus thiooxidans* |
| Lead-zinc mines 3 | Tailings | *Acidithiobacillus ferrooxidans* |
| | Ore | *Acidithiobacillus ferrooxidans*<br>*Desulfotomaculum nigrificans* |

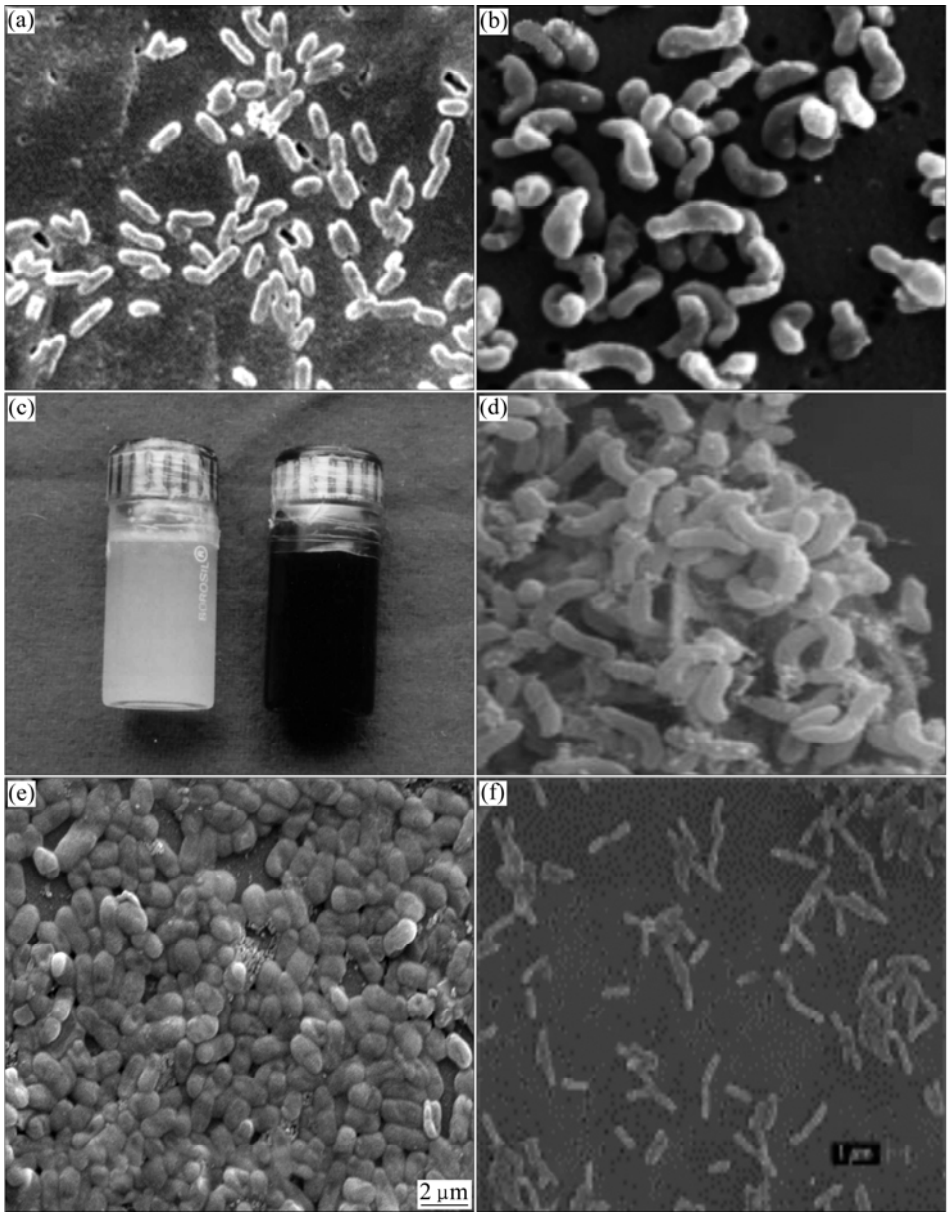



**Fig.1** Morphological features of mine-isolated bacteria: (a) *Acidithiobacillus ferrooxidans/thiooxidans*; (b) *Leptospirillum ferrooxidans*; (c) Black sulfide precipitation due to growth of SRB; (d) *Desulfovibrio* spp.; (e) *Desulfotomaculum nigrificans*; (f) *Thiomonas* spp.

### 4.2 Column and shake flask leaching

Column leaching and shake flask leaching tests over prolonged periods of time (about one year) were carried in the presence and absence of *Acidithiobacillus* group of bacteria using copper, lead-zinc and gold-bearing sulfide ores and their tailing samples. Typical results are illustrated in Figs.2−4.

As could be seen, the presence of *Acidithiobacillus ferrooxidans* accelerates acid generation and dissolution of copper, zinc and iron into the water medium. Probable mechanisms are given:

$$ZnS + 2O_2 \xrightarrow{A.\ ferrooxidans} Zn^{2+} + SO_4^{2-} \quad (4)$$

$$ZnS+2Fe^{3+} \rightarrow Zn^{2+}+S^0+2Fe^{2+} \quad (5)$$

$$2CuFeS_2 + 8.5O_2 + H_2SO_4 \xrightarrow{A.\ ferrooxidans} 2CuSO_4 + Fe_2(SO_4)_3 + H_2O \quad (6)$$

$$S^0+1.5O_2+H_2O \rightarrow SO_4^{2-}+2H^+ \quad (7)$$

Biooxidation of pyrite is given in Eqns.(1)−(3).

Similarly, biodisslution of arsenopyrite containing ores were determined and typical results are illustrated in Figs.5 and 6.

Column leaching studies depict a decrease in pH to around 4.5 accompanied by an increase in arsenic concentration to about 6 g/L in the presence of *A. ferrooxidans* in a period of 200 d. Arsenic leaching was lower in the absence of bacteria (around 1 g/L). On the other hand, kinetics of biodissolution of arsenopyrite was

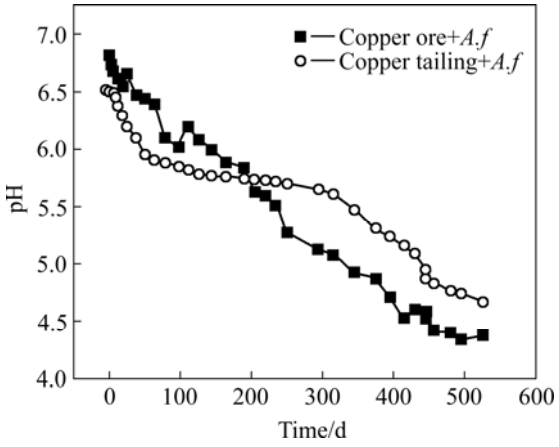



**Fig.2** Acid generation from copper ores and tailings in presence of *A. ferrooxidans*

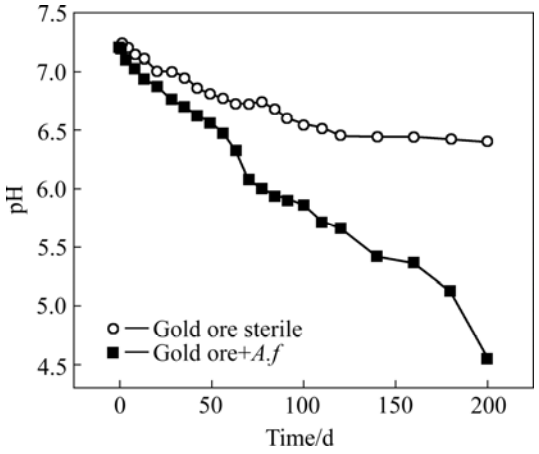



**Fig.5** Variation in pH due to activity of *A. ferrooxidans* in presence of arsenopyrite ores

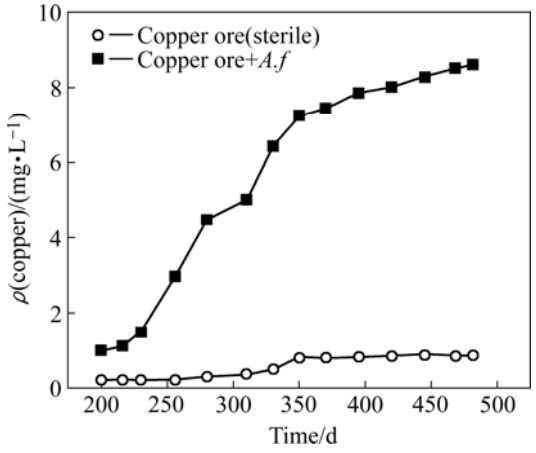



**Fig.3** Copper dissolution from chalcopyrite ore in presence and absence of *A. ferrooxidans*

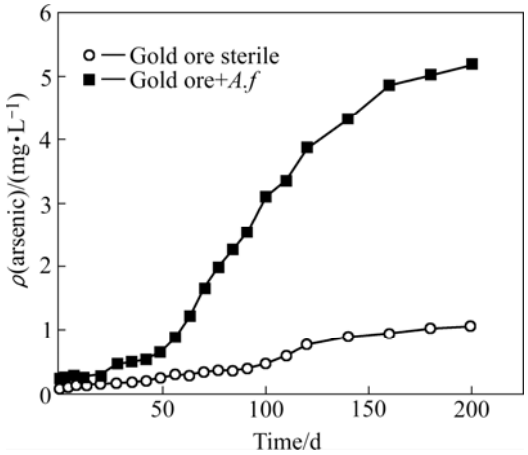



**Fig.6** Arsenic dissolution from arsenopyrite in presence and absence of *A. ferrooxidans*

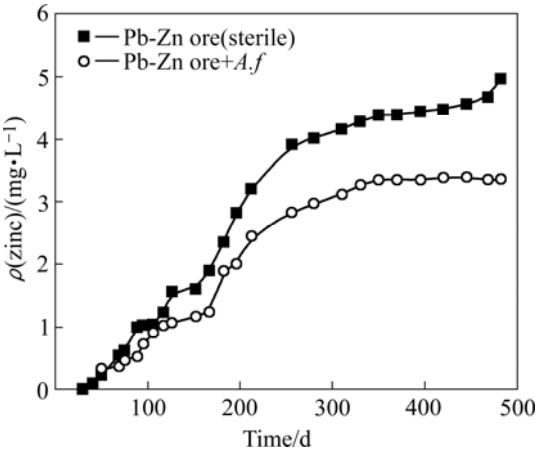



**Fig.4** Zinc dissolution from lead-zinc ore in presence and absence of *A. ferrooxidans*

faster in agitated shake flasks. The pH decreased to about 3.5 in a period of 280 d in inoculated flasks. Presence of bacteria enhanced the leaching of arsenic to about 12 g/L as compared with 2 g/L in the absence of bacteria in a period of 280 d.

Arsenopyrite decomposition can be expressed with the following reactions:

$$FeAsS+3.5O_2+H_2O \rightarrow Fe^{3+}+SO_4^{2-}+H_2AsO_4 \quad (8)$$

$$FeAsS+7H_2O \rightarrow Fe^{2+}+H_3AsO_3+11H^++11e+SO_4^{2-} \quad (9)$$

Presence of *Acidithiobacillus ferrooxidans* increased the rate of arsenopyrite oxidation in mine tailing piles catalyzing iron oxidation:

$$2Fe^{2+}+0.5O_2+2H^+ \xrightarrow{A.\ ferrooxidans} 2Fe^{3+}+H_2O \quad (10)$$

The presence of iron (Ⅲ) in solution further favours arsenopyrite oxidation:

$$FeAsS+13Fe^{3+}+8H_2O \rightarrow 14Fe^{2+}+SO_4^{2-}+13H^++H_3AsO_4 \quad (11)$$

Some of the Fe(Ⅲ) can react with the dissolved arsenate to precipitate as scorodite ($FeAsO_4 \cdot 2H_2O$).

Biodissolution of arsenopyrite is significantly influenced by the presence of pyrite and chalcopyrite due to galvanic interaction as seen in Fig.7.

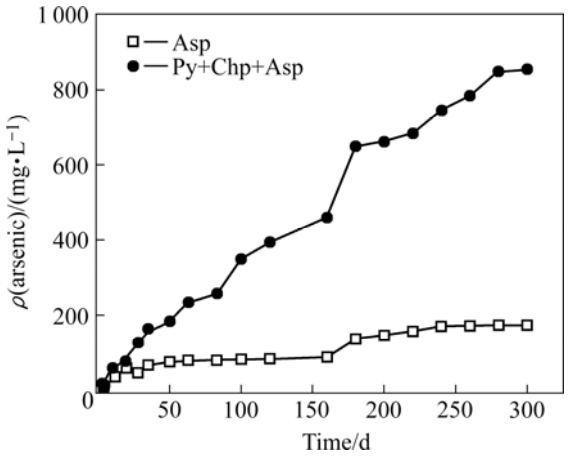
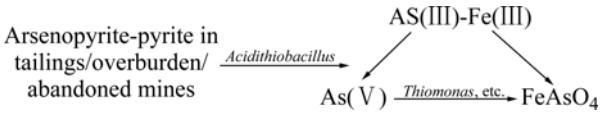



**Fig.7** Biodissolution of arsenopyrite in presence of pyrite and chalcopyrite

Presence of pyrite and chalcopyrite enhanced biodissolution of arsenopyrite in the presence of *A. ferrooxidans* due to galvanic effect. Both pyrite and chalcopyrite are nobler minerals compared with electrochemically active arsenopyrite.

Biooxidation of pyrite-arsenopyrite in the tailings/overburden/abandoned mines solubilizes arsenic preferentially to arsenite which could be further oxidized to arsenate by microorganisms such as *Thiomonas.* Similarly, ferrous ions released by biooxidation is oxidized to ferric ions which can tie up with arsenate forming iron precipitates as shown in Eqn.(12):




(12)

### 4.3 Bioremoval of copper, zinc and iron using sulphate reducing bacteria

Dissolved copper, zinc and iron in solutions could effectively be precipitated as their sulfides through interaction with Sulphate Reducing Bacteria(SRB). Mixed SRB cultures isolated from mines were used to demonstrate biodetoxification of copper, zinc and iron containing effluents. Typical results are shown in Fig.8.

Further tests on bioremoval of dissolved copper, zinc and iron were carried out in the presence of mine-isolated SRB at different initial concentrations of these metals. Use of SRB in metal sulfide precipitation when the above metals were present in different binary and ternary combinations was also examined. Typical results are illustrated in Tables 2−5.

Very efficient removal of copper, zinc and iron could be achieved from solutions containing varying concentrations of these metals through interaction with SRB cultures.

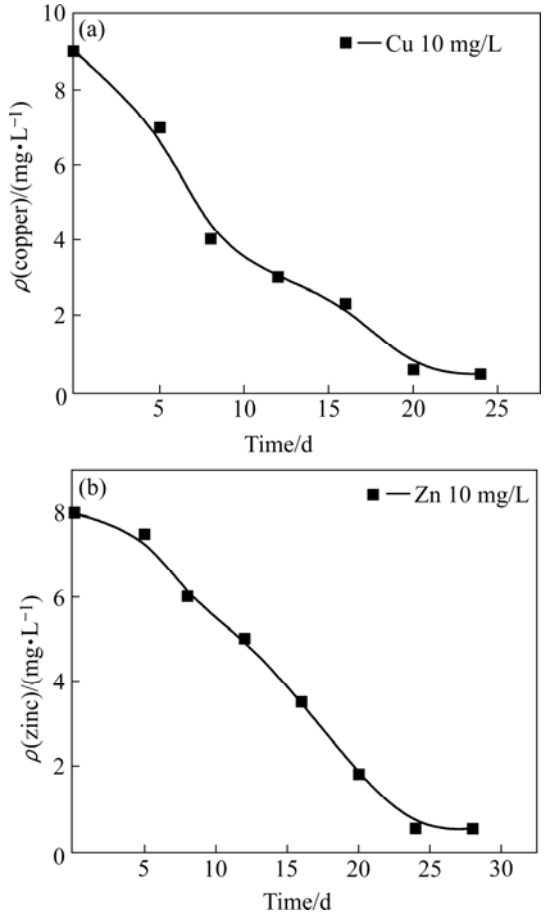



**Fig.8** Bioremoval of copper (a) and zinc (b)

**Table 2** Metal removal at neutral pH in 20 d at different initial concentrations

| Concentration/ (mg·L$^{-1}$) | 10 (Cu) | 40 (Cu) | 10 (Zn) | 40 (Zn) |
|---|---|---|---|---|
| Removal of metal/% | 96 | 89 | 80 | 75 |

**Table 3** Zinc and copper removal in 10 d when present together (10 m/L Zn+10 mg/L Cu)

| Removal of Zn/% | Removal of Cu/% |
|---|---|
| 88 | 80 |

**Table 4** Copper and iron removal in 5 d when present together (10 mg/L Cu + 30 mg/L Fe)

| Strain | Removal of Cu/% | Removal of Fe/% |
|---|---|---|
| SRB 1 | 86 | 85 |
| SRB 2 | 95 | 92 |

**Table 5** Metal removal in 10 d with copper, zinc and iron present together (10 mg/L Zn + 10 mg/L Cu + 10 mg/L Fe)

| Strain | Removal of Zn/% | Removal of Cu/% | Removal of Fe/% |
|---|---|---|---|
| SRB 1 | 79 | 57 | 37 |
| SRB 2 | 76 | 45 | 51 |

### 4.4 Biological remediation of arsenic

Biological arsenic removal was achieved through the following methods[10]. 1) Arsenic bioremoval through direct oxidation of the more toxic form of arsenic, namely, arsenite to arsenate using *Thiomonas* spp. and *Bacillus* spp. 2) The role of jarosites produced by *A. ferrooxidans* and *Leptospirillum ferrooxidans* in arsenic bioremoval through adsorption. 3) Role of SRP in precipitating dissolved arsenic as a sulfide. And 4) Arsenic adsorption onto SRP precipitates.

Arsenite oxidation was established in the presence of two bacterial isolates namely, *Thiomonas* spp. and *Bacillus* spp. Both the isolates exhibited efficient oxidation of arsenite in a period of 8 d.

Arsenic adsorption onto jarosites precipitates generated during growth of *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* was studied and typical results are presented in Table 6. Toxic arsenite could be effectively removed through this approach.

**Table 6** Arsenic adsorption onto jarosites generated by *Acidithiobacillus ferrooxidans* and *L. ferrooxidans*

| PH | Arsenic adsorption/% |
|---|---|
| 2.0 | 50 |
| 5.0 | 62 |
| 8.0 | 65 |
| 9.5 | 72 |

Sulphate Reducing Prokaryotes(SRP) can also be implicated in the bioremoval of heavy metals as illustrated already. Hydrogen sulfide produced during the growth of SRP aids in the precipitation of the metal ions as their metallic sulfides.

Under anaerobic conditions sulfate-reducing prokaryotes(SRP) reduce sulfate to hydrogen sulfide by using organic compounds or hydrogen as electron donor:

$$SO_4^{2-}+2CH_2O \rightarrow H_2S+2HCO_3 \quad (13)$$

Hydrogen sulfide precipitates metals as sulfides:

$$H_2S+M^{2+} \rightarrow MS(s)+2H^+ \quad (14)$$

where $M^{2+}$ is the metal, such as As(Ⅲ or Ⅴ).

The oxidation of electron donors produces alkalinity (e.g. $HCO_3^-$) which neutralizes acidic water:

$$HCO_3^-+H^+ \rightarrow CO_2(g)+H_2O \quad (15)$$

Bioremoval as As(Ⅲ) using SRB is illustrated in Fig.9. As could be seen, dissolved arsenite could be effectively removed through precipitation as sulfide and also through its adsorption onto the sulfide precipitates generated by SRB.

Sulphate reducing prokaryotes(SRP) reduce sulphate to sulphide:

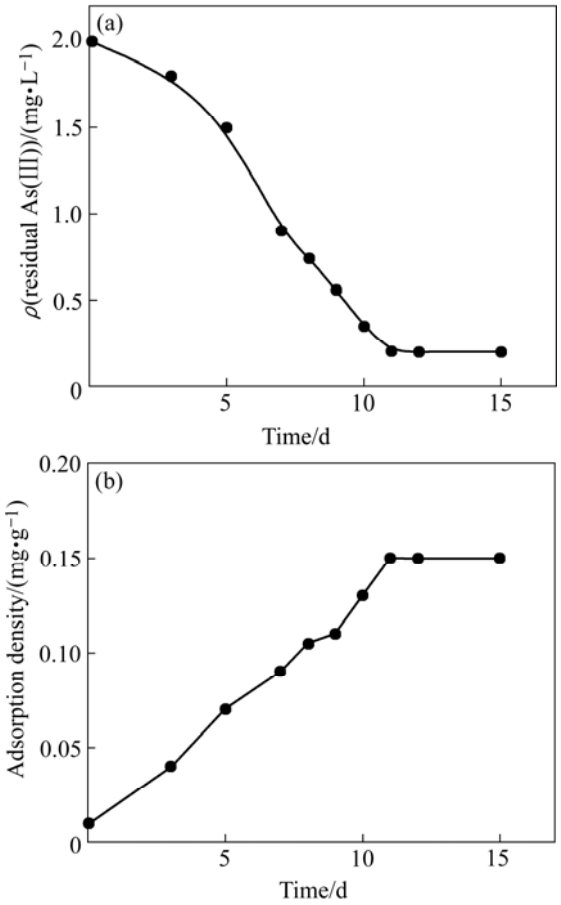



**Fig.9** Arsenic(Ⅲ) bioremoval using desulfotomaculum nigrificans: (a) Residual As(Ⅲ) concentration in presence of SRB culture; (b) Adsorption density in presence of precipitate as function of time

$$8Fe^{2+}+SO_4^{2-}+20H_2O \rightarrow 8Fe(OH)_3+14H^++H_2S \quad (16)$$

Arsenic precipitated by the sulphide as

$$2H_3AsO_4+5HS^- \rightarrow As_2S_5+3H_2O+5OH^- \quad (17)$$

$H_2S$ production and subsequent arsenic reduction/ precipitation using SRP can be accomplished at natural pH. Bacterial cell surfaces as well as the biogenic precipitated sulfides can also further adsorb dissolved arsenic. Ferric-arsenic precipitation can be used to dispose off remediated arsenic. *Acidithiobacillus* group of bacteria can be used to generate ferric and jarosite compounds on which arsenic is adsorbed/reacted and removed as inert sludge. Biooxidation of $As^{3+}$ to $As^{5+}$ (detoxification) through arsenic oxidizing bacteria such as *Thiomonas* is yet another remediation strategy as discussed above.

## 5 Conclusions

1) Various types of *Acidithiobacillus* group of bacteria responsible for acid mine drainage were isolated from different Indian mines.

2) Mechanisms and rate of dissolution of copper, zinc, iron and arsenic from copper ores, lead-zinc ores and gold-containing arsenopyrite ores and their tailings in the presence of *Acidithiobacillus ferrooxidans* were established through column leaching and shake flask leaching studies. Role of galvanic interactions in the biodissolution of arsenopyrite in the presence of pyrite and chalcopyrite is brought out.

3) Native bacteria such as *Thiomonas* and *Bacillus* spp. exhibited higher arsenic tolerance and were capable of oxidizing arsenite to arsenate.

4) Biological methods of remediation of acid mine drainage and bioremoval of copper, zinc, iron and arsenic are illustrated. Sulphate Reducing Prokaryotes could effectively precipitate all the above dissolved species as sulfides. Arsenic bioremediation can be effectively achieved through arsenite oxidizing bacteria such as *Thiomonas*, arsenic-precipitation and adsorption through SRP and through arsenite adsorption onto jarosites generated by *Acidithiobacillus* group of bacteria.

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