

## Original Research Article

# Arsenic bioleaching in medical realgar ore and arsenic-bearing refractory gold ore by combination of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*

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### Abstract

**Purpose:** To develop a novel biotechnological method for removing toxic arsenic from two kinds of representative arsenic-containing ores using different mixed mesophilic acidophiles.

**Methods:** Bioleaching of the two types of arsenic-containing ores by mixed arsenic-unadapted *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* or mixed arsenic-adapted cultures, were carried out. Arsenic bioleaching ratios in the various leachates were determined and compared.

**Results:** The results showed that the maximum arsenic leaching ratio obtained from realgar in the presence of mixed adapted cultures was 28.6 %. However, the maximum arsenic leaching ratio from realgar in the presence of mixed unadapted strains was only 12.4 %. Besides, maximum arsenic leaching ratios from arsenic-bearing refractory gold ore by mixed adapted strains or unadapted strains were 45.0 and 22.9 %, respectively. Oxidation of these two ores by sulfuric acid was insignificant, as maximum arsenic leaching ratios of realgar and arsenic-bearing refractory gold ore in the absence of any bacterium were only 2.8 and 11.2 %, respectively.

**Conclusion:** Arsenic leaching ratio of realgar and refractory gold ore can be enhanced significantly in the presence of arsenic-adapted mesophilic acidophiles.

**Keywords:** Adaptation, *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, Realgar, Arsenic-bearing refractory gold ore, Arsenic leaching ratio

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## INTRODUCTION

Realgar (As<sub>4</sub>S<sub>4</sub>, Xiong Huang in Chinese), as a common traditional Chinese mineral medicine, has been widely used for treatments of burns and scalds, insect-bites, abdominal pains, infantile convulsions, etc [1]. Recently, realgar has been successfully applied for treatments of refractory or relapsed acute promyelocytic

leukemia (APL) and chronic myelogenous leukemia (CML), and has been proved to be clinically effective [1-4]. Nevertheless, Wu et al. have observed that the solubilities of realgar in the stomach and intestinal fluids were only 0.10 and 0.40 %, respectively [5].

Poor water solubility and weak gastrointestinal absorption of coarse realgar powder impede its

clinical application [6]. Therefore, bioactive components of realgar leaching into aqueous solutions are necessary for its use in intravenous injections, instead of conventional oral administration. Our research group has presented a novel method for bioleaching of medical realgar ore that allowed much higher leaching rates of realgar than traditional leaching methods, increased absorption of the bioactive arsenic species, and avoided large dosages [1].

In addition, arsenic species with high concentrations are always detected in the leachates during bioleaching of gold concentrates and ores, especially in that of arsenic-bearing refractory gold ore.

Although, realgar and arsenic-bearing refractory gold ore are usually involved in two different fields, there is an identical that arsenic leaching ratio that constitutes a crucial factor in their bioleaching. Arsenic species leached from realgar are medically used as the major bioactive components, while arsenic species leached from arsenic-bearing refractory gold ore are by-products and need removing before cyanide extraction of gold. Furthermore, Zhang *et al* have proved that arsenic leaching ratio was much higher in the presence of bacterial mixed cultures than in the presence of a pure culture under the same condition [1].

*Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* are two of the most important bioleaching micro-organisms [7-10]. Mixed cultures of *A. ferrooxidans* and *A. thiooxidans* and some other acidophiles have been confirmed to be predominant bioleaching bacteria which can effectively oxidize and release metals or metalloids in the leachates during the bioleaching and bio-oxidation of sulfide ores, such as arsenic-bearing refractory gold ore [9,11-14]. However, only one strain of *A. ferrooxidans* and another strain of *A. thiooxidans* were employed in that report, and comparison of bioleaching behavior between mixed unadapted

cultures and adapted cultures was not investigated. Besides, several strains of *A. ferrooxidans* and *A. thiooxidans* have been isolated and adapted to yield high concentrations of inorganic arsenic species in our previous study; 15 rats of all known species of *A. ferrooxidans* BY-3 and TKY-2, *A. thiooxidans* JY and LYS were selected as four best performers which could tolerate up to 80 mM arsenite and 120 mM arsenate, respectively [15].

During bioleaching process, soluble arsenic species of higher concentrations may in turn inhibit bacterial bioactivities. Therefore, the micro-organisms that can resist high concentrations of soluble arsenic species are selected for use in bioleaching of arsenic-containing ores. In this study, a mixture of these four unadapted or adapted arsenic-resistant bacterial strains was employed to leach arsenic from realgar and arsenic-bearing refractory gold ore, respectively. A comparative study on arsenic leaching abilities among different treatments was discussed to investigate the efficiency of mixed cultures of four strains of *A. ferrooxidans* and *A. thiooxidans* to bio-oxidize and bioleach two kinds of different arsenic-containing sulfide ores.

## EXPERIMENTAL

### Micro-organisms and media

The experimental strains of *A. ferrooxidans* and *A. thiooxidans* employed in this study were described in Table 1. Isolation and identification of these acidophilic micro-organism strains have been reported in the previous work of our laboratory [16]. After adaptation to inorganic arsenic, all of them could resist soluble arsenic compounds of high concentrations [15].

In this study, both *A. ferrooxidans* and *A. thiooxidans* were cultured on modified Waksman medium [17] (Medium A) at 30 °C and 150 rpm, respectively.

**Table 1:** Description of sources and habitats of experimental strains of *A. ferrooxidans* and *A. thiooxidans*

Strain	Source and habitat
<b><i>Acidithiobacillus ferrooxidans</i></b>	
BY-3	Acid drainage from Baiyin copper mine, Gansu, China
TKY-2	Acid drainage from copper mine, Zhongtiao Mountain, Shanxi, China
<b><i>Acidithiobacillus thiooxidans</i></b>	
JY	Effluent of coal mine from Jingyuan County, Gansu, China
LYS	Soil sample of Lueyang copper mine, Shaanxi, China

The components of the medium were listed in detail (g/L):  $(\text{NH}_4)_2\text{SO}_4$  0.20,  $\text{K}_2\text{HPO}_4$  0.50,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.50, KCl 0.50,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  0.01,  $\text{Ca}_3(\text{PO}_4)_2$  0.50 and powdered sulfur 10, and the value of the pH was adjusted to 2.5 with dilute  $\text{H}_2\text{SO}_4$  solution. Before arsenic compounds were added into Medium A as required, the solution was sterilized by filtration through a 0.22  $\mu\text{m}$  pore size membrane filter.

However, another medium (Medium B) with a small amount of powdered sulfur and ferrous sulfate both supplemented as energy substrates for bacterial oxidative activities was then confirmed to be more suitable than Medium A when bio-oxidation experiments of realgar and arsenic-bearing refractory gold ore were respectively carried out. Medium B used for bio-oxidation experiments contained (g/L):  $(\text{NH}_4)_2\text{SO}_4$  0.20,  $\text{K}_2\text{HPO}_4$  0.50,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.50, KCl 0.50,  $\text{Ca}_3(\text{PO}_4)_2$  0.50, powdered sulfur 0.50,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  2.00, and the value of the pH was adjusted to approximate 2.0 with dilute  $\text{H}_2\text{SO}_4$  solution.

#### Description and preparation of realgar and arsenic-bearing refractory gold ore

Realgar used in this study was acquired from Changde City, Hunan Province, China, and Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES) analysis was conducted to quantitatively determine the major elemental abundances of this mineral drug. Before ICP-AES analysis, firstly, crude realgar sample was ground and sieved to a 200 mesh size. Secondly, crude sample was decomposed and digested, as explained below. Crude powdered realgar of 0.2 g was mixed equally with 10 mL deionized water, 5 mL  $\text{H}_2\text{O}_2$ , and 10 mL  $\text{HNO}_3 : \text{HCl}$  (1 : 1, v : v) solution in the beaker, then the mixture was heated to boiling point, incubated the mixture in the boiling water bath for about 1 h. Digested liquid was transferred into a 100 mL volumetric flask, flushed and complemented with deionized water to bring to volume, then diluted 100 times for determination of elemental contents by ICP-AES (Table 2).

Arsenic-bearing refractory gold ore used in this report was acquired from the low grade tailings of arsenic-containing refractory Pingding Gold Ore,

Zhouqu County, Gansu Province, China. Preparation of this crude sample was similar to that of realgar sample, except that decomposition and digestion were different from the former. The difference was that 0.5 g powdered gold ore was mixed equally with 10 mL  $\text{H}_2\text{O}_2$  and 20 mL  $\text{HNO}_3 : \text{HCl}$  (3 : 1, v : v) solution in the beaker, then the mixture was heated. The ICP-AES analysis results showed that elemental abundance of arsenic and gold was 11.29 % and 11.68 g/t, respectively.

#### Preparation of cell suspensions for bio-oxidation

Mixed culture of *A. ferrooxidans* BY-3, TKY-2 strains and *A. thiooxidans* JY, LYS strains (1 : 1 : 1 : 1) at the exponential phase of growth was inoculated into 500 mL Medium A and grown at 30 °C and 150 rpm. Until the value of optical density at 600 nm of the culture was observed to reach approximate 0.6. The culture was centrifuged at 4 °C and 2500 rpm for 5 min, and the non-oxidized powdered sulfur removed then re-centrifuged at 4 °C and 8000 rpm for 10 min; the supernatant discarded and collect the mixed bacterial cells collected. The washed cells were diluted with sulfuric acid (pH 2.0) twice, and then suspended in Medium B, with the mixed bacterial population in the suspensions reaching about  $1.0 \times 10^8$  cells/mL.

#### Arsenic bioleaching experiments

Arsenic bioleaching experiment of realgar and arsenic-bearing refractory gold ore was carried out in 250 mL Erlenmeyer flasks agitated on a rotary shaker at 150 rpm and 30 °C. Two different mixed cultures were involved in the experiments, i.e., one group with arsenic-unadapted mixed cultures of *A. ferrooxidans* BY-3, TKY-2 strains and *A. thiooxidans* JY, LYS strains (1 : 1 : 1 : 1) being the inoculum, the other group with arsenic-adapted mixed cultures being the inoculum. And non-inoculated group was selected as the negative control. 100 mL Medium B and 10 % (v/v) of the mixed cell suspensions were inoculated into every flask, each with 0.5 g fresh ground powdered ore (realgar or arsenic-bearing refractory gold ore) being added in, and the pH of the bioleaching system was adjusted to constantly approximate 2.0.

**Table 2:** Major elemental abundances in digested liquid of realgar by ICP-AES

As	Cu	Fe	Mg
73.71%	$5.147 \times 10^{-4}\%$	$92.46 \times 10^{-4}\%$	$51.62 \times 10^{-4}\%$
Pb	Ca	S	Si
$6.184 \times 10^{-4}\%$	1.87%	20.06%	3.59%

The process lasted for about 30 days. According to Yegorov *et al*, concentrations of Fe (II) and Fe (III) in the leachates were determined at the beginning of the experiment and every 5 days [18]. Besides, 1 mL of leachates were withdrawn for total contents of soluble arsenic species by ICP-AES analysis every 5 days as well, with each flask being supplemented simultaneously with equal volume of Medium B to keep the bioleaching system stationary. However, before ICP-AES analysis, filtration of the leachate samples through a 0.22  $\mu\text{m}$  pore size membrane filter was carried out.

### Statistical analysis

All the data were exhibited as mean values of three replicate determinations. Difference was considered to be significant when  $p < 0.05$ . Statistical analysis involved use of the OriginPro software package 8.5 (OriginLab. Corp.) and Design Expert software, version 8.0.5 (Stat-Ease Inc.).

## RESULTS

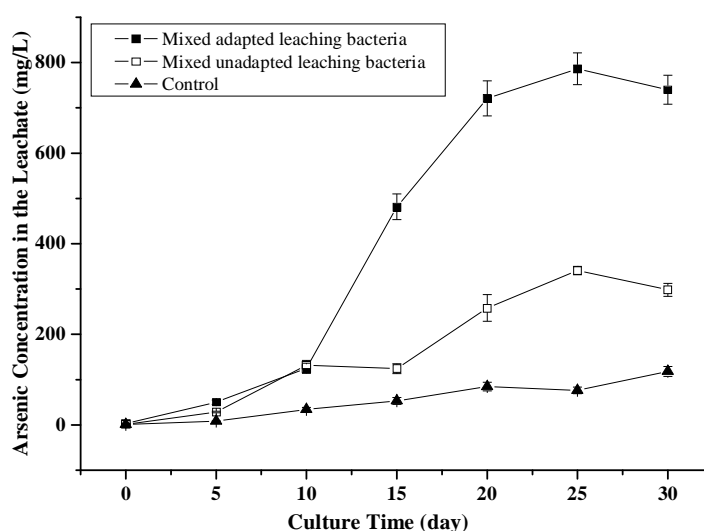
### Arsenic bioleaching of realgar

As shown in Fig. 1, during the whole observation, three maximum values of total concentrations of soluble arsenic species in the leachates of realgar corresponding to three different treatments were probably determined at the 25th day. According to these concentrations, arsenic bioleaching ratios in the presence of combinations of adapted bacteria and unadapted bacteria were 28.6 and 12.4 %, respectively,

whereas the negative control was only 2.8 % ( $p < 0.01$ ). Therefore, both *A. ferrooxidans* and *A. thiooxidans* could tolerate high concentrations of soluble arsenic compounds in the bioleaching environment after adaptations to arsenite and arsenate for a long time, and bio-oxidation rate by combination of the two acidophiles was much higher.

It was observed from the above data that bio-oxidation rate of realgar by mixed adapted bacteria was significantly higher than the mixed unadapted cultures.

Compared with non-inoculated negative control (proton as the major oxidant), arsenic leaching ratios in the presence of mixed cultures were higher, and very significant difference was indicated. It could be concluded that combination of acidophilic bioleaching bacteria played a key role in the bio-oxidation of realgar. Besides, it was noticed that bioleaching ratios of realgar in the presence of mixed cultures both decreased slightly after the 25th day. These phenomena were possibly due to two reasons, i.e., on one hand, when concentration of total soluble arsenic compounds in the leachate reached a high value, a small fraction of arsenic ion reacted with Fe (III) to form ferric arsenate precipitate and then was separated from the liquid phase; on the other hand, a part of arsenic ion leached from the solid ore adhered onto the surface of powdered sulfur and bacterial cells, which resulted in slowdown of bio-oxidation and bioleaching of realgar in the last few days.



**Figure 1:** Comparison of arsenic concentrations in the mixed bacterial leachates of realgar

### Oxidation rate of Fe (II) in the bioleaching of realgar

The aim of adding a small quantity of ferrous sulfate into the bioleaching system was to benefit ferrous-oxidizers. In addition, ferric ion which was transformed from ferrous ion by *A. ferrooxidans* could also oxidize realgar as another kind of oxidant. Sulfur-oxidizers were able to oxidize reduced inorganic sulfur in the bioleaching substrates, while the oxidized product sulfuric acid somehow accelerated the oxidation of ferrous ion (as described by Eqs 1 to 3). Similarly, oxidation of Fe (II) by mixed adapted cultures was faster than those by mixed unadapted cultures and the control. In detail, in the presence of combination of arsenic-adapted BY-3, TKY-2, JY and LYS, 76.5 % of Fe (II) was determined to be oxidized at the 10th day, and Fe (II) in the bioleaching system was totally oxidized at the 20th day.

Moreover, Fe (II) could not be detected in the leachates any more, which meant oxidation of realgar ore by Fe (III) was negligible in the last few days, so mixed bacterial cells and proton were considered to be superior to Fe (III) (Fig. 2). However, only 48 % of Fe (II) was observed to be transformed into Fe (III) in the presence of mixed arsenic-unadapted cultures at the 10th day, and Fe (II) was not completely oxidized until the 28th day. In the oxidation of Fe (II) in the

leaching flask was very slow and insignificant as only 12.5 % of Fe (II) was oxidized by the end of leaching experiment (Fig 2).

### Arsenic bioleaching of arsenic-bearing refractory gold ore

The results showed that the oxidation and decomposition rates of arsenic-bearing refractory gold ore were slightly slower than those of realgar ores, owing to differences of structures and mineral constituents between the ores. In particular, arsenic bioleaching ratios of arsenic-bearing refractory gold ore were observed to be relatively low (e.g., only about 10 %) during the first half of the bioleaching process. There was no obvious difference of arsenic bioleaching ratios in the presence of mixed unadapted bacteria and in the presence of adapted bacteria (Fig. 3). However, during the second half of the experiment, arsenic bioleaching ratio by the combination of adapted cultures was vastly superior to that of unadapted cultures, as maximum arsenic bioleaching ratio by the former was 45.0 %, while the ratio by the latter was only 22.9 % ( $p < 0.01$ ). Besides, arsenic leaching and oxidation of arsenic-bearing refractory gold ore in the control were negligible, as maximum arsenic leaching ratio by the abiotic factor was just 11.2 %, which was far below the level of microbiological treatments (Fig 3).

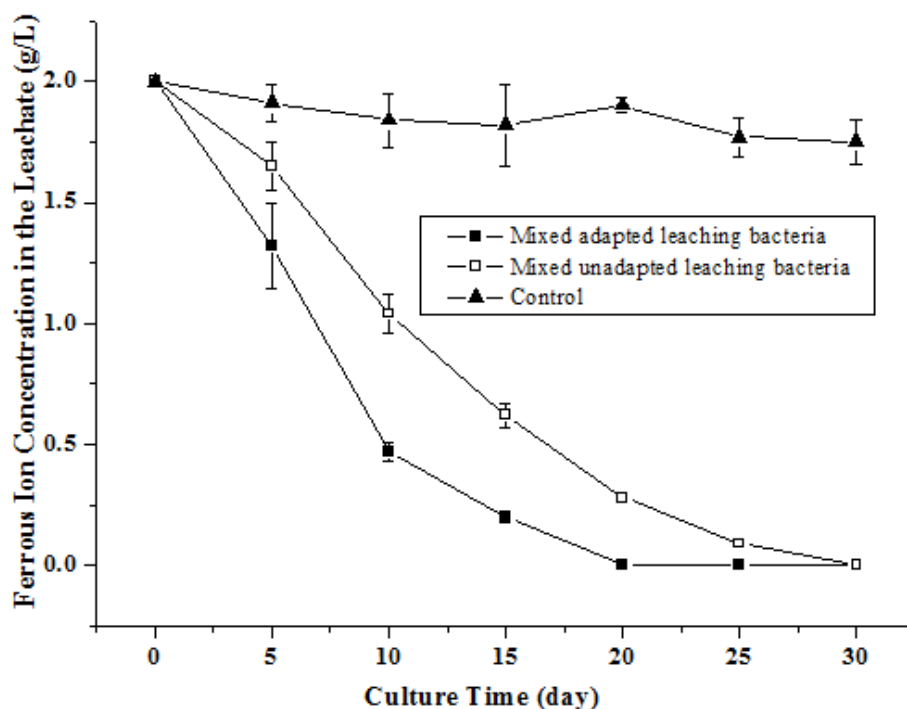
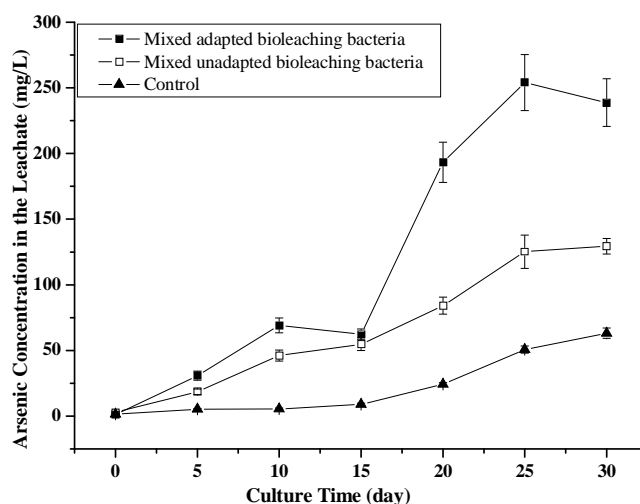
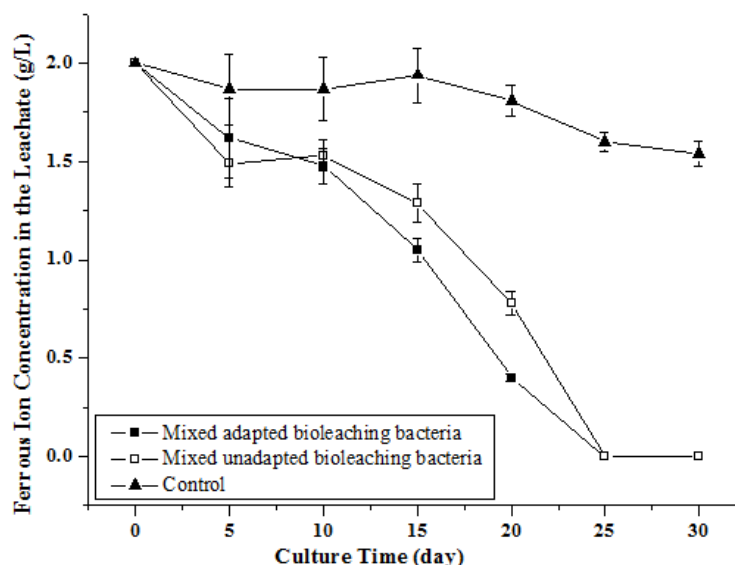


Figure 2: Comparison of ferrous ion concentrations in mixed bacterial leachates of realgar



**Figure 3:** Comparison of arsenic concentrations in the mixed bacterial leachates of arsenic-bearing refractory gold ore



**Figure 4:** Comparison of ferrous ion concentrations in the mixed bacterial leachates of arsenic-bearing refractory gold ore

### Oxidation rate of Fe (II) in the bioleaching of arsenic-bearing refractory gold ore

The overall variation trend of oxidation rate of Fe (II) in the bioleaching of arsenic-bearing refractory gold ore was analogous to that of realgar. Energy for bacterial growth was derived from elemental sulfur, ferrous sulfate and powdered ore in the system. It was noticed that Fe (II) oxidation rates of combinations of adapted cultures and unadapted cultures were both relatively high from the 10th day to the 25th day. Compared with Fe (II) oxidation rate in the presence of mixed unadapted bacteria, the presence of mixed adapted bacteria the oxidation

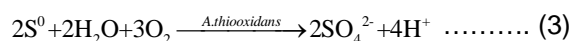
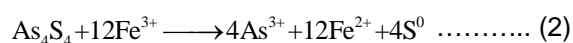
rate was higher by 2.5 – 19 %; while ferrous ions have been completely oxidized by the end of 25th day. In the negative control, oxidation of Fe (II) by proton and soluble oxygen in the liquid was incomplete, and oxidation rate was very low (Fig 4).

### DISCUSSION

Realgar is arsenic-containing sulfide ore, widely applied in the field of medicine. Arsenic-bearing refractory gold ore is common and accounts for a large proportion of gold ores widely used to bioleach and recover precious metal gold. These two natural ores were selected to elaborate how

much and how fast arsenic ions could be leached from the raw ores into the leachates with different combinations of microbial sulfide oxidizers.

In this bioleaching system, energy for bacterial growth was derived from oxidation of trace elemental sulfur, Fe (II) and realgar itself. With bioleaching experiments, more and more bacterial cells adhered onto the surface of powdered sulfur and realgar ore, which ore was gradually oxidized and decomposed in the presence of both proton and mixed acidophilic bacteria. The elemental arsenic ions which was transformed into arsenite, arsenate or other soluble arsenic species, were released into the leachates from powdered realgar. In addition, it was observed that oxidation rate of realgar and leaching ratio of arsenic ions were significantly enhanced by Fe (III); which was oxidized by *A. ferrooxidans* from trace Fe (II) supplemented in the bioleaching system. Generally, the oxidation of realgar ore can be described in three equations (Eqs 1 to 3).



The behaviors and changes of bio-oxidation of realgar in this paper were all consistent with the previous work, even though arsenic bioleaching ratio and oxidation rate of Fe (II) reported here were a little bit lower. As far as the maximum arsenic bioleaching ratio from realgar was concerned, the ratio reached at 56% reported previously by another team [1], while the maximum value obtained at the 25th day in this work was 28.6%. One possible reason for explaining this difference was that experimental materials were neither from the same batch nor from the same mining region. As a consequence, physicochemical properties of raw realgar ores used in different reports were different.

When bio-oxidations of pyrite and arsenopyrite were in progress, Breed and other researchers found that with increasing concentrations of As (III) and As (V) bioleached from the arsenic-containing ores, the bioleaching bacterial activities was strongly inhibited by these arsenic species, as enhancement of arsenic bioleaching ratios was hindered [19,20]. Nevertheless, similar situations had not occurred in our bioleaching experiments of arsenic-bearing refractory gold ore in the presence of mixed adapted cultures.

The four bacteria strains employed in this study had been adapted to high concentrations of inorganic arsenic compounds and their oxidative activities would not be inhibited by increasing concentrations of soluble arsenic species in the leachates. Therefore, in the second half of bioleaching process, arsenic bioleaching ratio reached a relatively high concentration in the presence of mixed adapted cultures, which was clearly superior to the concentrations in the presence of mixed unadapted cultures or in the absence of any bacterium. Furthermore, the abilities of combination of four inorganic arsenic-adapted strains of *A. ferrooxidans* and *A. thiooxidans* to bio-oxidize and bioleach these two sulfide ores were both significantly enhanced than that in the presence of mixed arsenic-unadapted cultures.

As a consequence, *A. ferrooxidans* BY-3, TKY-2 and *A. thiooxidans* JY, LYS strains, isolated and identified by our laboratory appeared most valuable and predominant bacteria in bioleaching industry. However, the specific bioleaching technology still needs further study.

## CONCLUSION

The developed biotreatment presented higher arsenic removing efficiency than traditional chemical treatment, especially with the aid of mixed adapted indigenous cultures. Arsenic leaching ratio of realgar and refractory gold ore can be enhanced significantly in the presence of arsenic-adapted mesophilic acidophiles.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

No conflict of interest associated with this work.

## CONTRIBUTION OF AUTHORS

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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