International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2736-1756 Vol. 15 (12), pp. 001-007, December, 2016. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Identification of *Thiobacillus thiooxidans* CGMCC 10329 and fundamental application in bioleaching as part of a synergic bacterial consortium

Hongfei Zhu[1*] and Xuesong Zhao[2]

[1]College of Environmental Science and Engineering and [2]Mining School of Liaoning Technical University No. 47 Zhonghua Road, Fuxin, Liaoning 123000, China.

Accepted 12 October, 2016

Bioleaching has become increasingly important in commercial gold extraction because of its economic benefits. *Thiobacillus thiooxidans* (also known as *Acidithiobacillus thiooxidans*) is an important participant in synergic bioleaching processes. In this study, a novel strain was isolated from an underground coal mine, identified and named *Thiobacillus thiooxidans* CGMCC 10329. A consortium of microorganisms including *T. ferrooxidans* DSM14882, *Leptospirillum ferrooxidans* CCTCC AB207038 and *T. thiooxidans* CGMCC 10329 was used to leach four gold ores and four sulfur-containing coal samples; we then determined gold recovery rates and removal rates of elements. The content that may be harmful to the environment, such as sulfur and arsenic contained in the ores, was investigated. The synergic leaching results indicated that the maximum deprivation rates of carbon, sulfur and arsenic were respectively 59.91%, 67.21%, and almost 100%. Bioleaching pretreatment markedly improved the gold recovery rate by 10.7% to 20% in subsequent cyanidation, compared with traditional extraction operations. The average sulfur removal rate by synergic leaching of the four coal samples was 35.4%. This synergic leaching method, to some extent, broadens gold ore resources and benefits the environment by reducing the impact of coal burning.

Keywords: *Thiobacillus thiooxidans*; synergy; bioleaching; recovery rate; sulfur; deprivation

INTRODUCTION

Bioleaching applied in metal recovery from mineral ores has progressed steadily in the past two decades. Compared with conventional metallurgical processes, bioleaching methods are able to turn commercially unattractive minerals into valuable ones (Rawlings and Kusano, 1994). This technology establishes its economic advantages through low energy consumption, environmental benefits, low cost and simple operation. A variety of chemolithotrophic and heterotrophic microorganisms are responsible for the solubilization of metals from sulfide minerals or sulfur elimination from coal (Siezen and Wilson, 2009, Dopson and Johnson, 2012). *Thiobacillus ferrooxidans*, *Leptospirillum ferrooxidans* and *T. thiooxidans*, regarded as the major participants in bioleaching, form a consortium of microorganisms used for the industrial recovery of gold or coal desulfuration (Yin et al., 2014, Valdes et al., 2008, Fujimura et al., 2012).

T. thiooxidans (also called *Acidithiobacillus thiooxidans*) inhabits extremely acidic environments and creates favorable acidic conditions for the growth of ferrous iron oxidizing bacteria (Yin et al., 2014, Falco et al., 2003). This bacterium plays an important role in bioleaching processes. *T. thiooxidans* contributes to elemental sulfur oxidation and has the ability to remove a sulfur layer from mineral surfaces, which is otherwise

*Correspondence author: hongfei.zhu@yahoo.com

a barrier to the dissolution of the mineral ore (Lizama and Suzuki, 1988). Complete genomes are available for *T. thiooxidans* strains ATCC 19377^T, A01 and Licanantay, which paved the way for studying the leaching ability of *T. thiooxidans* globally (Travisany et al., 2014, Valdes et al., 2011, Yin et al., 2014). Much attention has been paid to the capability of consortia in which *T. thiooxidans* is involved (Hong et al., 2013). The optimal bacterial consortium of different species for a given metal is frequently of interest (Latorre et al., 2016). Gene expression in bacterial consortia and its relevance to leaching have been investigated (Liu et al., 2011). However, the leaching synergy of *T. thiooxidans*, *T. ferrooxidans* and *L. ferrooxidans* on mineral ores and sulfur-containing coal has rarely been reported. Does a consortium including *T. thiooxidans* have more capacity in mineral bioleaching than any two of the leaching bacteria, or bioleaching by *T. ferrooxidans* alone?

In this work, experiments were performed to establish the synergy of *T. thiooxidans*, *T. ferrooxidans* and *L. ferrooxidans*. Firstly, a strain of *T. thiooxidans* was isolated from an underground coalmine and characterized to better understand the microbial diversity and adaptive characteristics of bioleaching. Secondly, a mixture of *T. ferrooxidans*, *L. ferrooxidans* and the novel strain of *T. thiooxidans* was used to pretreat gold ores and sulfur-containing coal to investigate subsequent levels of gold recovery by cyanidation, the elimination of hazardous elements, and coal desulfurization. The results indicated that this consortium is synergically effective in the bioleaching of gold ores and in coal desulfurization.

MATERIALS AND METHODS

Minerals

Gold ore powder (Samples 1, 3 and 4) were all refractory and obtained from Liaoning Paishanlou Gold Stock Company Limited. The particle diameter of the gold ore powders was approximately 0.074 mm and >90% of the particles were of this size. Carbon and sulfur in Sample 1 and arsenic in sample 2 (gold concentrate) were the target elements to be leached out. Samples 3 and 4 were used exclusively to determine the gold recovery rate by cyanidation after pretreatment by bioleaching.

Coal samples Aiyou, Xingfu, AYYM and HDXL were all collected from Fuxin Mining Group, Liaoning, China. Coal sample AYYM had the highest sulfur content. Table 1 summarizes data on the original samples.

Bacteria

The microbes of the consortium used in this work were *T. ferrooxidans* DSM14882, *L. ferrooxidans* CCTCC AB207038 and *T. thiooxidans* CGMCC 10329. *T. thiooxidans* CGMCC 10329 was isolated and characterized in the present study. *T. ferrooxidans* and *L. ferrooxidans* were cultured in 9K medium: $(NH_4)_2SO_4$ (3.0 g/l), K_2HPO_4 (0.5 g/l), $MgSO_4{\cdot}7H_2O$ (0.5 g/l), $Ca(NO_3)_2$ (0.01 g/l), $FeSO_4{\cdot}7H_2O$ (10 g/l) (filter sterilized) and KCl (0.01 g/l) (Hirt and Vestal, 1975). The pH was 2.0.

T. thiooxidans was cultured in Starkey medium: $MgSO_4{\cdot}7H_2O$ (0.5 g/l), $CaCl_2$ (0.25 g/l), K_2HPO_4 (3.5 g/l), $(NH_4)_2SO_4$ (0.3 g/l), $FeSO_4{\cdot}7H_2O$ (0.01 g/l), and sulfur powder (10.0 g/l) (Cook, 1964). The pH was also adjusted to 2.0.

Isolation and characterization of *T. thiooxidans* CGMCC 10329

A water sample was collected from a pit located 350 m below sea level in Qinghemen Coal Mine, Fuxin, Liaoning, China. Five milliliters of the pit water were immediately added to 100 ml Starkey medium in an Erlenmeyer flask. The flask was then shaken at 100-150 rpm after 3 d of standing at 37°C. The shaken cultivation lasted for 7-9 days until a white cloud appeared at the bottom of the culture (Starosvetsky et al., 2013).

Bacterial solution (1 ml) was spread thoroughly on solid Starkey medium; the culture dish was kept at 37°C for 8-10 days until small white colonies grew. A single colony was picked into liquid Starkey medium for enrichment. Solid and liquid cultures were repeated alternately with the aim of purification.

Genomic DNA from the putative *T. thiooxidans* isolate was extracted as described previously (Zhu et al., 2012). Gram-staining and PCR targeting the 16S rRNA gene were used to detect *T. thiooxidans* (primers Tt13: ATCACTGGGCGTAAAGGG and Tt2: AACCCAACATCTCACGACAC).

Bioleaching

Development of synergic leaching system

The density of all the pulps for leaching was 10% (w/v) with the starting pH 2.0. In the synergic leaching system, the proportions of *T. ferrooxidans*: *L. ferrooxidans*: *T. thiooxidans* by number in the bacterial inoculation were approximately 2:2:1. The total bacterial density at the start was about 1×10^4-1×10^5 CFU/ml. In an attempt to provide optimum growth conditions for the leaching bacteria, gold ore samples 1, 2, 3 and 4 were leached in unsterilized 9K liquid medium. Likewise, to make sulfur contained in coal powder fully usable by the bacteria, Aiyou, Xingfu, AYYM and HDXL coal samples were added to distilled water.

The minimum amount of all the processed gold ore samples was ≥400 g for target element detection and recovery calculation. The pH value was monitored using a Multiparameter Monitor for Water Quality (YSI556MPS, Geotech Environmental Equipment Inc., USA) every 24 h. If the pH value in bioleaching exceeded 3.5, it was immediately readjusted to 2 with H_2SO_4.

Controls only differing in that they contained only *T. ferrooxidans* instead of the bacterial mixture were set in parallel for each sample for the purpose of comparison with synergic bioleaching; *T. ferrooxidans* was present in these controls at 1×10^4-1×10^5 CFU/ml.

Flasks containing leaching pulps were cultured at 37°C with shaking at 120 rpm for 9-11 days.

Sample treatment

The gold ore powders after the leaching process were simply collected by centrifugation and washed three times with distilled water. The powder was baked to dryness at 80-100°C. The processed coal powder was handled with freeze-thawing and sonication steps in alkali and acid to remove salts, such as jarosite, adhering to the coal particles. First, the coal powder was frozen in water at −80°C for 1 h and then thawed in air; this process was repeated once and would remove salts from the grain surface. Second, the coal was treated by sonication (Ultrasonic Both, KQ-300GVDV, Kunshan, China) in 1 M NaOH and 1 M HCl solutions, respectively, for 1 h. Finally, the powder was washed three times with distilled water and baked to dryness at 60°C.

Gold cyanidation extraction and gold recovery rates

This process was finished by Liaoning Paishanlou Gold Stock Company Limited, China. Gold cyanidation, a commonly used process, is a metallurgical technique for extracting gold from gold ores by converting the metal to a water-soluble coordination complex (Yap and Mohamed, 2007). Sodium cyanide, in the form of a very dilute solution, is used to dissolve and separate gold from ore. Chemically, this rather simple reaction is: $4Au + 8(NaCN) + O_2 + 2H_2O \rightarrow NaAu(CN)_2 + 4NaOH$.

The extraction process was as follows: the to-be-pretreated mineral powders, Samples 3 and 4 (350 g each), were added to distilled water to make pulp with a concentration of 40%-42% (w/v) and the pulp was adjusted to pH 10-12 with lime. Then, approximately 0.0049 g sodium cyanide, based on average dosage (14 kg/ton), was added to the pulp to form a gold-cyanide complex. Subsequently, activated charcoal (15 g/l) was added to the pulp to absorb the gold complex. Electrolysis was carried out to desorb the gold from the activated charcoal in conditions of electric tension 3 V, electric current 2000-4000 A and temperature 100-150°C. The electrolysis step took 6-8 h in alkaline conditions (with 5% NaOH). The gold complex-containing mud gathered at the negative pole with a density of 30%-50%. Finally, smelting refinement at 1250-1300°C was required to reach 99.9% purity of gold. The ratio of the obtained pure gold to the gold contained in the sample was regarded as the recovery rate.

Detection of removal efficiency

The carbon and sulfur components in gold ore Sample 1 were measured using a Mono Pipe High Temperature Carbon Furnace (SK2-2-13, China). This detection was also carried out by Liaoning Paishanlou Gold Stock Company Limited, China. The arsenic in Sample 2 was detected by ICP-MS (Inductively coupled plasma mass spectrometry) by Qingdao S&S Chemicals Analysing and Testing Co. Ltd. The total sulfur contained in the coal powder samples was detected with an Infrared Sulfur Detector (SC-114DR) by Northeast University, China.

The deprivation rates of the targeted elements were calculated using the formula:

$$R_{dE} = \frac{E_O - E_P}{E_O}$$

where R_{dE} (%) is the deprivation rate in gold ore or coal powder; “E” indicates the target element (carbon, sulfur or arsenic); and “o” and ”p” indicate “original” and “processed” samples, respectively.

RESULTS AND DISCUSSION

Characterization of *T. thiooxidans* CGMCC 10329

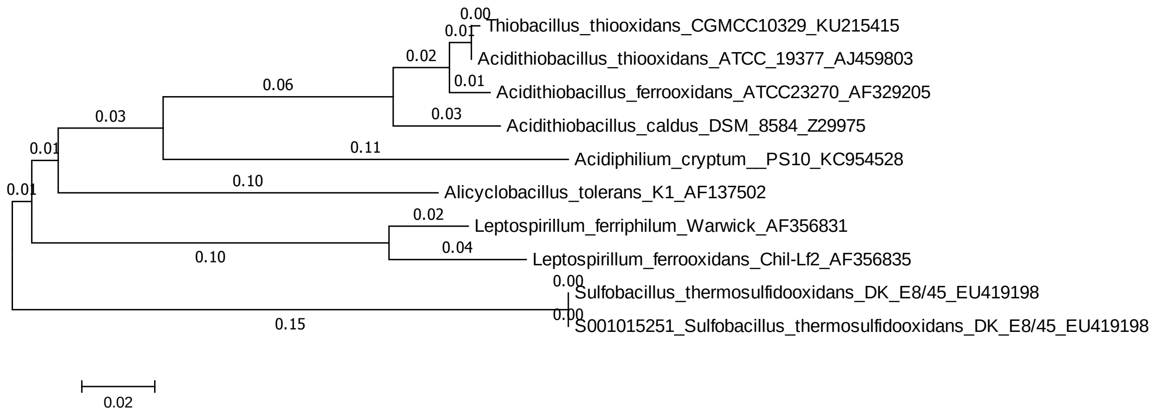
Gram-staining of the isolated bacterium was negative. PCR with primers Tt13 and Tt2 targeting the 16S rRNA gene produced an amplicon of 553 bp, as expected for *T. thiooxidans*. The amplicon was sequenced and the sequence has been deposited in GenBank with accession number KU215415. BLAST results of the sequence (Figure 1) exhibited 99% identity and 99% similarity with the 16S rRNA gene sequence of *T. thiooxidans* ATCC 19377^T. The newly isolated strain was therefore assigned as *T. thiooxidans*; it has been deposited in the China General Microbiological Culture Collection Center (CGMCC) with accession number CGMCC 10329.

A phylogenetic tree based on the sequences of 16S rRNA genes of related bioleaching bacteria was constructed using the neighbor-joining algorithm to demonstrate their evolutionary relationships with *T. thiooxidans* CGMCC 10329 (Figure 2). It was observed that *T. thiooxidans* CGMCC 10329 and *Acidibacillus thiooxidans* ATCC 19377 were in the same clade and the two strains could reasonably be considered to be the same species based on their 16S rRNA sequences. *A. ferrooxidans* ATCC 23270 was phylogenetically the closest relative in the same group and *A. caldus* DSM 8584 was next.

Removal efficiency and recovery rate

The carbon content in gold Sample 1 synergically leached by three bacteria (SLBTB) and leached solely

Figure 2. Phylogenetic tree of bioleaching bacteria based on comparative 16S rRNA gene sequences and constructed using the neighbor-joining method.



by *T. ferrooxidans* (LSBTF) was 0.85% and 1.37% respectively, compared with 2.12% in the original sample. These results corresponded to carbon deprivation rates of 59.91% and 35.37% respectively. Meanwhile the sulfur content was 0.4% and 0.79% following SLBTB and LSBTF treatment respectively, compared with 1.22% in the original sample, corresponding to sulfur deprivation rates of 67.21% and 35.24%. All the above data are shown in Table 1. The carbonaceous material that preferentially absorbs gold and gold-cyanide complexes during gold extraction was greatly reduced by the synergic bacterial leaching and therefore the preg-robbing effect of the carbon during cyanidation was lessened. Thus, the carbonaceous gold ores could be leached synergically to improve the final gold production. The sulfur deprivation data showed that both pyrite and reduced sulfur compounds were easily decomposed in the synergic leaching system, which would reduce the quantity of SO_2 gas resulting from ore smelting (Hong et al., 2013).

The gold recovery rates from Samples 3 and 4 following SLBTB-treatment reached 96% and 79% respectively, compared with 81.3% and 72.3% from the LSBTF portion, and 76% and 68.3% using the traditional smelting method without bioleaching pretreatment before cyanidation (Table 1). Moreover, the optimal sodium cyanide dosage decreased from an average 14.0 kg/ton in the conventional extraction method to 9.21 kg/ton for the same sample LSBTF-treated and 7.9 kg/ton following SLBTB-treatment. The decreased use of sodium cyanide in synergic gold leaching is of great signifcance to organisms and environment due to its toxicity.

The rate of arsenic removal from gold concentrate (Sample 2) was almost 100% (original arsenic content 12.26%) (Table 1). The residual arsenic in Sample 2 was as little as 5.1 mg/kg (SLBTB) or 7.4 mg/kg (LSBTF) (Table 1). The arsenic traces left could be totally neglected in the process of gold extraction. These results demonstrate that arsenic was easily oxidized into soluble arsenate in both the synergic leaching system and on treatment with *T. ferrooxidans* only.

On the whole, both the deprivation rates and the recovery rates resulting from the synergic leaching were superior to those on leaching with *T. ferrooxidans* only. The consortium of *T. ferrooxidans*, *L. ferrooxidans* and *T. thiooxidans* interact and cooperate with each other metabolically, which effectively dissolved contaminating minerals during bioleaching. Thus, this synergic leaching method not only increased the precious metal production, but also decreased the environmental pollution due to arsenic or SO_2. Moreover, this method could enhance commercially unattractive mineral ores, which would increase the availability of mineral resources worldwide and help cope with current crises in demand. The leaching bacteria presumably easily decompose the mineral ores packaged by pyrite or arsenopyrite by destroying their crystal forms. Therefore, the valuable gold inside the ores was uncovered and able to react readily with cyanide for solubilization and extraction.

As for the coal samples, both the original Aiyou and Xingfu coal samples contained 0.9% sulfur. The sulfur content of the Aiyou sample following SLBTB and LSBTF treatment was, respectively, 0.43% and 0.84% (Table 1). The sulfur content of the Xingfu sample following these treatments was 0.35% and 0.83%, respectively. Therefore, the maximum observed sulfur deprivation rates in Aiyou and Xingfu coal were 52.2% and 61.1% respectively, whereas the deprivation rates of Samples HDXL and AYYM were 20.4% and 7.91% by the same treatment (Table 1). Although the desulfurization rates were in line with the finding that synergic leaching was advantageous, the differing rates

Figure 1. BLAST result for 16S rRNA gene of T. thiooxidans CGMCC 10329.

Acidithiobacillus thiooxidans strain ATCC 19377 16S ribosomal RNA gene, partial sequence

Sequence ID: ref|NR_044920.1|Length: 1470 Number of Matches: 1

Related Information

Range 1: 557 to 1041GenBankGraphics

Score	Expect	Identities	Gaps	Strand
883 bits(478)	0.0	483/485(99%)	2/485(0%)	Plus/Plus

```
Query  1     GTCTGTCGTG-AATCCCCGGGCTC-ACCTGGGAATGGCGGTGGAAACCGGTGTACTAGAG  58
             |||||||||| ||||||||||||| |||||||||||||||||||||||||||||||||||
Sbjct  557   GTCTGTCGTGAAATCCCCGGGCTCAACCTGGGAATGGCGGTGGAAACCGGTGTACTAGAG  616

Query  59    TATGGGAGAGGGTGGTGGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAA  118
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  617   TATGGGAGAGGGTGGTGGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAA  676

Query  119   CATCAGTGGCGAAGGCGGCCACCTGGCCCAATACTGACGCTGAGGCACGAAAGCGTGGGG  178
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  677   CATCAGTGGCGAAGGCGGCCACCTGGCCCAATACTGACGCTGAGGCACGAAAGCGTGGGG  736

Query  179   AGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGAATACTAGATGTTTG  238
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  737   AGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGAATACTAGATGTTTG  796

Query  239   GTGCCAAGCGTACTGAGTGTCGTAGCTAACGCGATAAGTATTCCGCCTGGGAAGTACGGC  298
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  797   GTGCCAAGCGTACTGAGTGTCGTAGCTAACGCGATAAGTATTCCGCCTGGGAAGTACGGC  856

Query  299   CGCAAGGTTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTT  358
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  857   CGCAAGGTTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTT  916

Query  359   TAATTCGATGCAACGCGAAGAACCTTACCTGGGCTTGACATGTCTGGAATCCTGCAGAGA  418
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  917   TAATTCGATGCAACGCGAAGAACCTTACCTGGGCTTGACATGTCTGGAATCCTGCAGAGA  976

Query  419   TGCGGGAGTGCCCTTCGGGGAATCAGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTG  478
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  977   TGCGGGAGTGCCCTTCGGGGAATCAGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTG  1036

Query  479   TCGTG  483
             |||||
Sbjct  1037  TCGTG  1041
```

between samples may partly be due to imbalanced growth of leaching bacteria or the coal quality. It is inferred that the sulfur is bonded tightly by carbon atoms or impeded by other elements in samples HDXL and AYYM. Further research is needed to determine the differences in coal samples that lead to these large differences in sulfur deprivation rates. Overall, however, the synergic processing method described here may be applied to the desulfurization of high-sulfur coal or other fuel, which would decrease the emission of SO_2 into the atmosphere in the course of coal burning.

The initial added amount of bacteria of each species in the consortium was known. However, the numbers of bacteria of each species in the medium and late stages of sample treatment remain ambiguous. The widely differing rates of sulfur deprivation in various coal

Table 1. Original and leached content of gold ore and coal samples.

	Content or item (%)	Sample 1			Sample 2			Sample 3			Sample 4		
Gold ore samples		O	T	S	O	T	S	O	T	S	O	T	S
	Carbon	2.12	0.85	1.37									
	Sulfur	1.22	0.40	0.79									
	As				12.26%	7.4[a]	5.1[a]						
	Recovery rate							76	96	81.3	68.3	79	72.3
Coal samples	Content or item (%)	Aiyou			Xingfu			AYYM			HDXL		
		O	T	S	O	T	S	O	T	S	O	T	S
	Sulfur	0.90	0.43	0.84	0.90	0.35	0.83	1.91	1.52	1.77	1.39	1.28	1.30
	Deprivation rate(%)		52.2	6.67		61.1	7.78		20.4	7.33		7.91	6.47

[a]Unit: mg/kg.
“O” stands for “original” (i.e. the original sample); “T” for ”three” in the process **s**ynergically **l**eached **b**y **t**hree **b**acteria; “S” for “solely” in the process **l**eached **b**y **s**olely ***T**. **f**errooxidans*.

samples may depend on the precious composition of bacterial consortium used for treatment or perhaps be influenced by the changing growth ratio of the bacteria used. Further optimization is required to achieve maximum bacterial growth, maximum yields of metal extraction and optimal removal of contaminating elements. Biological parameters including bioleaching time, proportions of the three bacteria and growth factors (temperature, pH and aeration) should be assessed to optimize the bioleaching. More powerful microbes for bioleaching should also be exploited for the recovery of gold and contaminant removal; therefore, more powerful microorganisms of bioleaching interest should be isolated, identified and genomically sequenced. The optimal combination of microbes would result in maximum leaching. Different target metals should be handled with various optimized leaching microbes and methods (Vardanyan et al., 2015). Sensitive and specific molecular methods, such as real-time PCR and microarrays, should be developed to probe the leaching bacteria during the whole process, so that the bacterial enumeration can be adjusted at any time to achieve the optimal leaching (De Wulf-Durand et al., 1997, Bond and Banfield, 2001).

CONCLUDING REMARKS

In this work, a mixture of three species of moderately thermophilic bacteria (*T. ferrooxidans*, *L. ferrooxidans* and *T. thiooxidans*) was used for the efficient bioleaching of gold ores and coal. Pretreatment by synergic bioleaching improved the gold recovery rate in subsequent cyanidation. Copper, molybdenum, zinc, manganese, uranium, and other metals can all be extracted using a similar approach.

Like arsenic, poisonous metals (such as mercury, cadmium and lead) in solid compounds in mineral ores can be transformed into soluble salts by bioleaching (Ko et al., 2013, Lee et al., 2015, Zhang et al., 2015). The harmful ions in the liquid phase can easily be collected and dealt with as precipitates before discharge into the environment. Hence, the recovery of metals from both smelting and bioleaching becomes a benign process with the aid of this preprocessing of poisonous metals.

Coal desulfurization is a useful tool to enable the use of high-sulfur coal that is otherwise prohibited by strict environmental laws because of excessive emission of sulfur dioxide. Coal with lower sulfur content is environmentally beneficial.

ACKNOWLEDGEMENTS

We thank Mark Dopson and Jun Wang for kindly providing the bacteria *T. ferrooxidans* DSM14882 and *L. ferrooxidans* CCTCC AB207038 respectively. We also thank Paishanlou Gold Stock Company Limited for the help with detection of the gold recovery rate. This work was supported by grants from Education Department of Liaoning Province, China (LJYL042) and the National Natural Science Foundation of China (31270479).

REFERENCES

Bond PL, Banfield JF (2001). Design and Performance of rRNA Targeted Oligonucleotide Probes for in Situ Detection and Phylogenetic Identification of Microorganisms Inhabiting Acid Mine Drainage Environments. Microbial ecology, 41(2)**:** 149-161.

Cook TM (1964). Growth of *Thiobacillus Thiooxidans i*n Shaken Culture. J Bacteriol, 88**:** 620-623.

De Wulf-Durand P, Bryant LJ, Sly LI (1997). PCR-mediated detection of acidophilic, bioleaching-associated bacteria. Appl Environ Microbiol, 63(7)**:** 2944-2948.

Dopson M, Johnson DB (2012). Biodiversity, metabolism and applications of acidophilic sulfur-metabolizing microorganisms. Environ Microbiol, 14(10)**:** 2620-2631.

Falco L, Pogliani C, Curutchet G, Donati E (2003). A comparison of bioleaching of covellite using pure cultures of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* or a mixed culture of *Leptospirillum ferrooxidans* and A*cidithiobacillus thiooxidans*. Hydrometallurgy, 71(1)**:** 31-36.

Fujimura R, Sato Y, Nishizawa T, Oshima K, Kim SW, Hattori M, Kamijo T, Ohta H (2012). Complete genome sequence of *Leptospirillum ferrooxidans* strain C2-3, isolated from a fresh volcanic ash deposit on the island of Miyake, Japan. J Bacteriol, 194(15)**:** 4122-4123.

Hirt WE, Vestal JR (1975). Physical and chemical studies of *Thiobacillus ferroxidans* lipopolysaccharides. J Bacteriol, 123(2)**:** 642-650.

Hong FF, He H, Liu JY, Tao XX, Zheng L, Zhao YD (2013). Comparison analysis of coal biodesulfurization and coal's pyrite bioleaching with *Acidithiobacillus ferrooxidans*. TheScientificWorldJournal, 2013**:** 184964.

Ko MS, Park HS, Kim KW, Lee JU (2013). The role of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* in arsenic bioleaching from soil. Environmental geochemistry and health, 35(6)**:** 727-733.

Latorre M, Cortes MP, Travisany D, Di Genova A, Budinich M, Reyes-Jara A, Hodar C, Gonzalez M, Parada P, Bobadilla-Fazzini RA, Cambiazo V, Maass A (2016). The bioleaching potential of a bacterial consortium. Bioresource technology, 218**:** 659-666.

Lee E, Han Y, Park J, Hong J, Silva RA, Kim S, Kim H (2015). Bioleaching of arsenic from highly contaminated mine tailings using *Acidithiobacillus thiooxidans*. Journal of environmental management, 147**:** 124-131.

Liu Y, Yin H, Zeng W, Liang Y, Baba N, Qiu G, Shen L, Fu X, Liu X (2011). The effect of the introduction of exogenous strain *Acidithiobacillus thiooxidans* A01 on functional gene expression, structure and function of indigenous consortium during pyrite bioleaching. Bioresource technology, 102(17)**:** 8092-8098.

Lizama HM, Suzuki I (1988). Bacterial leaching of a sulfide ore by *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*: I. Shake flask studies. Biotechnol Bioeng, 32(1)**:** 110-116.

Rawlings DE, Kusano T (1994). Molecular genetics of *Thiobacillus ferrooxidans*. Microbiological reviews, 58(1)**:** 39-55.

Siezen RJ, Wilson G (2009). Bioleaching genomics. Microb Biotechnol, 2(3)**:** 297-303.

Starosvetsky J, Zukerman U, Armon RH (2013). A simple medium modification for isolation, growth and enumeration of *Acidithiobacillus thiooxidans* (syn. *Thiobacillus thiooxidans*) from water samples. J Microbiol Methods, 92(2)**:** 178-182.

Travisany D, Cortes MP, Latorre M, Di Genova A, Budinich M, Bobadilla-Fazzini RA, Parada P, Gonzalez M, Maass A (2014). A new genome of *Acidithiobacillus thiooxidans* provides insights into adaptation to a bioleaching environment. Research in microbiology, 165(9)**:** 743-752.

Valdes J, Ossandon F, Quatrini R, Dopson M, Ds. H (2011). Draft genome sequence of the extremely acidophilic biomining bacterium *Acidithiobacillus thiooxidans* ATCC 19377 provides insights into the evolution of the *Acidithiobacillus* genus. Journal of Bacteriology, 193(24)**:** 7003-7004.

Valdes J, Pedroso I, Quatrini R, Dodson RJ, Tettelin H, Blake R, 2nd, Eisen JA, Holmes DS (2008). *Acidithiobacillus ferrooxidans* metabolism: from genome sequence to industrial applications. BMC Genomics, 9**:** 597.

Vardanyan A, Stepanyan S, Vardanyan N, Markosyan L, Sand W, Vera M, Zhang R (2015). Study and assessment of microbial communities in natural and commercial bioleaching systems. Minerals Engineering.

Yap CY, Mohamed N (2007). An electrogenerative process for the recovery of gold from cyanide solutions. Chemosphere, 67(8)**:** 1502-1510.

Yin H, Zhang X, Li X, He Z, Liang Y, Guo X, Hu Q, Xiao Y, Cong J, Ma L (2014). Whole-genome sequencing reveals novel insights into sulfur oxidation in the extremophile *Acidithiobacillus thiooxidans*. Bmc Microbiology, 14(1)**:** 1-14.

Zhang G, Chao X, Guo P, Cao J, Yang C (2015). Catalytic effect of Ag(+) on arsenic bioleaching from orpiment (As(2)S(3)) in batch tests with *Acidithiobacillus ferrooxidans* and *Sulfobacillus sibiricus*. J Hazard Mater, 283**:** 117-122.

Zhu H, Wang Q, Wen L, Xu J, Shao Z, Chen M, Reeves PR, Cao B, Wang L (2012). Development of a multiplex PCR assay for detection and genogrouping of *Neisseria meningitidis*. Journal of clinical microbiology, 50(1)**:** 46-51.