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Pyrite Desulfurization Under Variable Initial pH Conditions Using Extreme Thermophilic Bacteria

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To obtain an effective bacterium involved in biodesulfurization at elevated temperatures and investigate its pyritic sulfur removal behaviors at variable initial pH, an extreme thermophile from Tengchong hot spring, China, was isolated and adapted, followed by four groups of pyrite column bioleaching with 15% (v/v) bacterial inoculation at pH 1.0, 1.5, 2.0 and 2.5, respectively. Experiments show that the extreme thermophilic bacteria is spherical or oval-shaped and obligately autotrophic in iron-sulfur environment. The column leaching experiments show that 15% bacterial inoculation leads to a rapid pyrite oxidization after a short bacterial adaptation at pH 1.0~2.5. At initial pH 2.0, 21.7% biodesulfurization rate and 39.2g/L soluble iron concentration are obtained after 36 days leaching. Initial pH plays a key role in bacterial adaptation and activity, and enables the oxidization of Fe(II) and thiosulfate intermediates, therefore controls the desulfurization rate. At lower initial pH, Fe(III) ions are more susceptible to formation of secondary iron-bearing minerals than sulfate ions in high iron concentration environment. Results show that this isolate is amenable to pyritic sulfur degradation under high acidity and high temperature conditions.

Key words: Biodesulfurization, Initial pH, Extreme Thermophile, Precipitation, Mineral Morphology.

Microbial leaching is an increasingly important alternative to extract metals from sulfide ores, especially for those with low grade and waste rocks[1,2]. Pyrite is the most abundant sulfide mineral that frequently associated with other sulfide ores[3]. The existence of pyrite can positively affect the kinetics of bioleaching due to the galvanic effect that occurs between different minerals with dissimilar rest potentials. With a higher rest potential compared to other sulfides, pyrite tends to be cathodically protected in the aqueous systems. Other sulfides with lower rest potentials are therefore dissolved at an accelerating rate[4]. However, the potential hazard of spontaneous combustion increases once the sulfur content exceeds 15% within the heap or stack. It is a large part due to the heat generation caused by oxidation reactions of sulfide minerals[5].

Conventional methods to avoid the combustion of sulfide ores are mostly carried out base on external causes control, such as reduction of moisture content, particle segregation and oxygen isolation. Over the past few years, more and more researchers have noticed that microbial leaching is an effective internal way to prevent sulfide combustion, because of its capability to transfer the reductive solid sulfides to soluble sulfates. As early as 1988, Rai and Reyniers[6] studied the different desulfurization roles by P. *putida* and *P. aeruginosa* of the genus *P. seudonmons* on laboratory scale, they found that *P. putida* was capable to reduce the pyritic sulfur by 75% in coals and lignites. Prayuenyong[7] later

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summarized the species of mesoacidophilic bacteria that involved in coal sulfur degradation, and proposed the mechanism and optimal conditions for microbial desulfurization. More recently, Yin *et al.*,[8] conducted flask experiments to test effects of bacteria inoculations, solution pH, pulp densities and particle sizes on biodesulfurization by At. *Ferrooxidans*, they announced that the spontaneous combustion tendency of sulfides decreased from class I to class III after bioleaching.

The preceding studies were mostly realized by using acidophilic mesophilic and moderately thermophiles under ambient temperature conditions. Some ore or waste rock heaps are of great heights due to geological or operation cost considerations. The heat generated at the bottom or middle part of the heap therefore becomes formidable to diffuse outside. Moreover, the high acidity caused by pyritic reactions negatively affect the microbial metabolism. Hence, the implement of bioleaching in ambient temperature environment is challenged by harsh conditions within these heaps. Ruan and co-workers[9] introduced acidophilic mesophilic and moderately thermophilic bacteria to leach secondary sulfide ores containing 5.8% pyrite, they found the temperature increased to 70 °C within the heap and the pH decreased to around 0.8 during bioleaching, thereby bacterial generation and metal extraction were both inhibited.

Temperature and pH values are invariably key factors that govern microbial leaching and desulfurization behaviors in elevated temperature environments. Fortunately, extreme thermophilic archaea and bacteria have been reported effective in bioleaching[10,11]. Extreme thermophiles are advantageous in thermostability, salinity tolerance and acid resistance. It indicates an improvement in kinetics of biodesulfurization process in high temperature and acidity environment.

The objective of this work is to conduct bioleaching of pyrite using an extreme thermophile, with an emphasis on the effect of initial pH on biodesulfurization at elevated temperatures. Firstly, an acidic extreme thermophilic strain isolated from a hot spring was cultured and adapted to understand its biological nature. Secondly, four groups of column leaching were performed with initial pH values range uniformly from 1.0 to 2.5. Additionally, variations of pyrite leaching and bacterial activity were discussed throughout the microbial leaching process.

MATERIALS AND METHODS

Mineral characteristics

Ore samples were provided by Xinqiao Mine in Anhui province, China. The raw ores were ground to 3~5 mm in diameter and sealed in plastic bags to prevent mineral oxidation prior to experiments. The ore comprised predominantly pyrite and quartz gangue. Mass percentages of FeS_2 and SiO_2 were 86% and 14%, respectively.

Enrichment and isolation

Samples were collected from Tengchong hot spring, China, where the water temperature was 90 °C and pH was 2.5. Before bioleaching, the strain was cultured on an orbital shaker with a rotary speed of 50 r/min at 70 °C, and the initial acidity was adjusted to pH 2.0 with 1 M sulfuric acid. When the medium pH decreased to about 1.0 after 7~10 days, 20 mL of bacteria-containing solution was transferred to a 350-mL flask filled with 180 mL culture media for successive cultivation. Likewise, a total of five generations of bacterial enrichment were sequentially carried out. The basal culture consisted of 3.0 g/L $(NH_4)_2SO_4$, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L K_2HPO_4, 0.1 g/L KCl, 0.01 g/L $Ca(NO_3)_2$, 1.0 g/L S0 and 0.01 %(w/V) yeast extract.

Bacterial adaptation

The enriched bacteria were adapted with sulfide ore powders to test their sulfur oxidation ability. Sulfides involved 0.5% chalcopyrite concentrate and 0.5% pyrite concentrate (particle size, d90=20 μm), respectively The adaptation, with 10% bacteria inoculation, were successively cultured similar to bacterial enrichment method for 7 days.

Column bioleaching experiments

The column bioleaching experiments were conducted in four plexiglass columns, 50 mm in inner diameter and 500 mm high. A 5-mm heat preservation interlayer between inwall and outwall was linked to thermostatic water bath that operated in the range 67~73 °C. Initial pH values of the feed solution for Column 1 to Column 4 were adjusted to 1.0, 1.5, 2.0 and 2.5, respectively. Each column was charged with 1600 g ores, above which lied 200 g gravel particles. The bed heights of pyrite and gravels were approximately 350 mm and 40 mm, respectively. Prior to leaching, ore samples were washed with distilled water to remove impurities. The feed solution contained 15% bacteria medium, and flowed through the ore bed at a rate of 150 L/m^2/h by gravity. The pregnant leaching solutions (PLS) in collective containers were pumped backward to the feed container in order to maintain the leaching process.

Statistical Analysis

During the course of the bioleaching experiment, 5 mL PLS from each column was collected for determination of solution pH, redox potential and ion concentrations (Fe(II), total iron (TFe) and SO_4^{2-}). The sampling and the evaporation were compensated with sterile distilled water. The pH and redox potential were monitored by SX-620 pH monitor with Ag/AgCl as reference electrode at room temperatures. Concentrations of TFe and Fe^{2+} were determined by titration with sodium diphenylamine sulfonate as an indicator. The Fe(III) concentration was calculated by subtracting the Fe(II) concentration from the TFe concentration. Additionally, the SO_4^{2-} concentrations were directly measured by DX-120 ion chromatograph.

RESULTS AND DISCUSSION

Isolation and adaptation of the culture

The cultures are spherical or oval-shaped, and they tend to absorb to the substrate surface singly or in pairs. Microscopical studies and 16S rRNA gene sequencing have not been carried out yet, thus its classification and species stands unclear. The enrichment and adaptation experiments show that this strain can grow in a wide acidity range from pH 1.0 to pH 5.0 at 70 °C, with an optimum at pH 2.0. This acidophilic microorganism is obligately autotrophic, and derives growth energy from the oxidation of ferrous iron and elemental sulfur. One of the key characteristic is that its growth and reproduction is not retarded even when the total iron concentration reaches 38.9 g/L.

The ability of the isolates to oxidize sulfide ores is evaluated. Solution pH values decrease at a certain speed due to the increase of sulfate ions (Fig. 1), which indicates that the strain successfully degraded the chalcopyrite and pyrite concentrates. As expected, the strain exhibits good growth and its amenability to sulfides. The appearance of the controlled sample keeps colorless throughout the experiment (Fig. 2(a)), whereas the color turns to blue-green in the presence of chalcopyrite (Fig. 2(b)) and yellow in the presence of pyrite (Fig. 2(c)). The adaptation tests indicate copper and iron are effectively extracted by extreme thermopiles.

Fe leaching under variable initial pH conditions

The Fe(II) and Fe(III) concentrations increase gradually before day 20 and exhibit a degree of stability afterwards (Fig. 3). It can be calculated that the highest TFe concentration is 39.2g/L in Column 3 (Fig. 4(c)), whereas the lowest TFe concentration is 24.6 g/L in Column 2 (Fig. 4(b)).

Since pyrite is an acidic insoluble mineral, the conversion extent of Fe(II) to Fe(III) depends on bacterial activity. The bacteria in logarithmic phase is inoculated to feed medium prior to leaching, it therefore exerts a rapid oxidization of pyrites after a short adaptation. However, the increase of soluble iron concentrations is limited after day 20, indicating a suppression of bacterial activity. When bacterial growth reaches a stationary phase, pyrite dissolution rate is hindered mainly due to a decrease in bacterial metabolism and iron-oxidizing ability in energy-depleted columns. On the other hand, precipitation forms and adheres to the ore surface with time. These compounds then become a barrier for intra-particle diffusion of leaching solution and released iron ions.

Redox potentials in PLS are 570~589 mV at the beginning and increase slightly until day 16, then fluctuate around 600 mV within a narrow range after day 20 (Fig. 4). In general, redox potentials correspond to the Fe(III)/Fe(II) ratio according to nernst equation12. A high Fe(III)/Fe(II) ratio is conducive to the increase of redox potential, whereas potential over 500 mV has been reported a positive range accelerating the pyrite leaching[13]. Bacterial oxidization ability decreases in its stationary phase after day 20, thus lowers the Fe(III)/Fe(II) ratio and solution redox potential. Redox potentials in four groups are relatively lower compare with those using acidophilic mesophilic bacteria in spite of initial pH values[14]. This is in agreement with Petersen and Dixon's observation that high operation temperature is accountable for lowering solution potential[15]. High temperatures partially govern the Fe(III) adsorption and ions (Fe^{3+}, K^+, and SO_4^{2-}) precipitation on the particle surface, and therefore reduce the Fe(III)/Fe(II) ratio.

Desulfurization rates under variable initial pH conditions

Biodesulfurization rates increase at different speeds throughout the bioleaching process, and final rates reach to a range of 12.5% (Column 4) to 21.7% (Column 3) after 36 days (Fig.4). This variation is basically in the same pattern as total soluble iron concentrations. The biologically catalyzed oxidation of acid-insoluble pyrite follows an thiosulfate mechanism[16]. Under acidic conditions, the Fe(II)-oxidizing bacteria oxidize the ferrous iron to the ferric iron (Eq.(1)), which is the only oxidant available for biochemical reaction in aqueous system. The pyritic sulfur is released to solution due to microbial corrosion and then turns to thiosulfate intermediates. However, the thiosulfate is unstable in acidic media[17] and thus, oxidized to stable sulfate by bacteria (Eqs.(2~3)). Initial pH values play a key role in bacteria adaptation and oxidation activity, hence results in a disperse among desulfurization rates. Similar with bacterial adaptation experiments, the strain is stimulated with greatest oxidation capacity at initial pH 2.0 in Column 3.

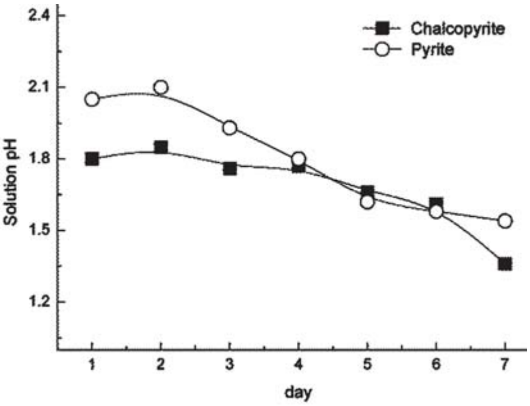

Fig.1. Solution pH evolutions during bacterial adaptation experiments

$2Fe^{2+}+0.5O_2+2H_+ \rightarrow 2Fe^{3+}+H_2O$ ..(1)

$FeS_2+6Fe^{3+}+3H_2O \rightarrow S_2O_3^{2-}+7Fe^{2+}+6H^+$ ..(2)

$S_2O_3^{2-}+8Fe^{3+}+5H_2O \rightarrow 2SO_4^{2-}+8Fe^{2+}+10H^+$ ..(3)

Comparison of Fe and S leaching

The final biodesulfurization rate is partially limited by ion adsorption and precipitation. Assume that the thiosulfate is completely converted to the sulfate, then the productive molar concentration ratio of SO_4^{2-}/TFe will be 2 according to the molecular formula of FeS2. Ratios of SO_4^{2-}/TFe display in the range of 2.07 (Column 1) to 1.72 (Column 4), and gradually decrease with increasing initial pH values (Fig. 5). These values show good accordance with the ideal value, indicating the extreme thermophilic bacteria is capable of thiosulfate oxidization. In the presence of high iron concentration (28.4g/L) at lower pH, the SO_4^{2-}/TFe ratio in Column 1 shows that Fe(III) ions are more

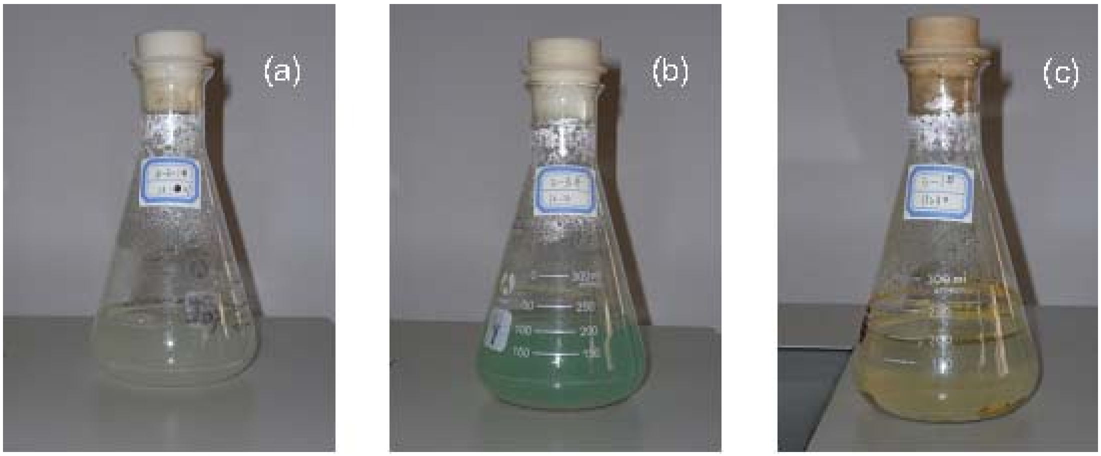

Fig. 2. The appearance after bacterial adaptation for 7 days: (a) controlled sample, (b) sample with chalcopyrite and (c) sample with pyrite

susceptible to precipitate than sulfate ions.

Sulfide mineral morphology

The SEM images of pyrite in Column 3 before and after bioleaching show distinct surface morphologies (Fig. 6). Particle before leaching has a smooth and integrate surface with regular crystals distribution. As the biodesulfurization process, the extreme thermophilic culture continues to catalyze the pyrite dissolution, leaving irregular pits in the porous structure.

Jarosite is the main composition of the precipitation that distributes inconsistently on the ore surface, where few elemental sulfur is also detected. As a common hydroxysulfate in bacteria

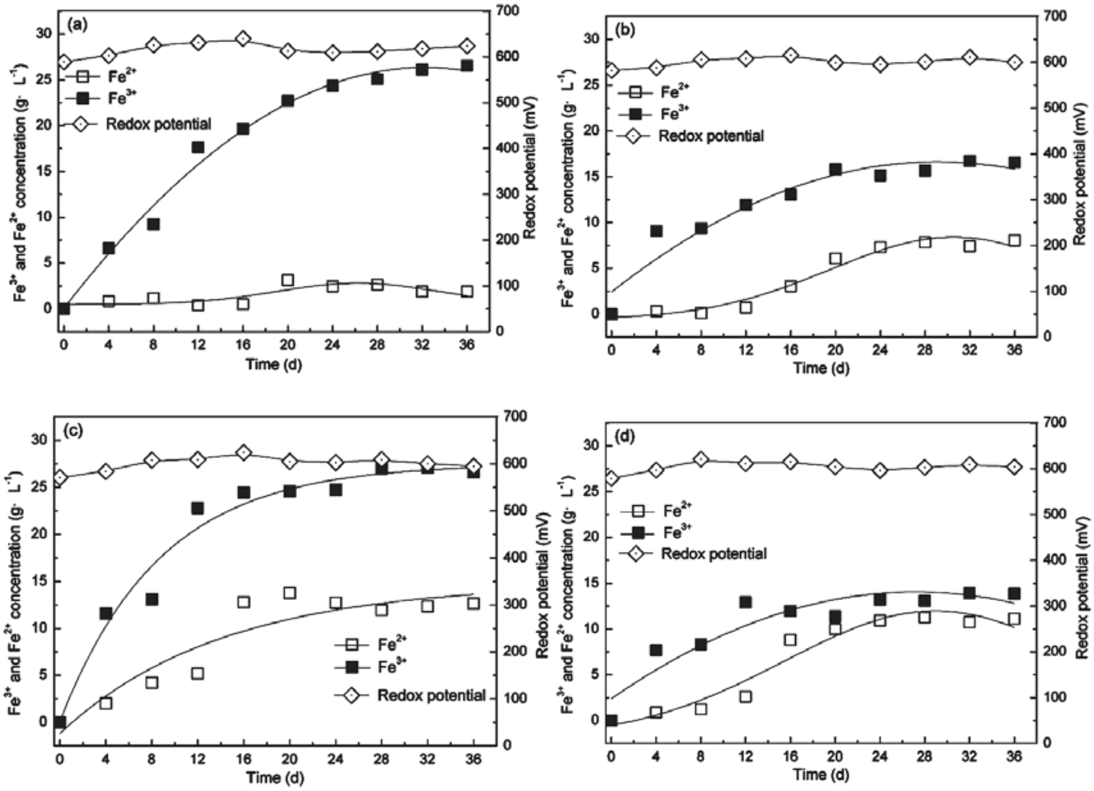

Fig.3. Redox potential, Fe(II) and Fe(III) concentration variations throughout the bioleaching process: (a)initial pH=1.0; (b)initial pH=1.5; (c)initial pH=2.0; (d)initial pH=2.5

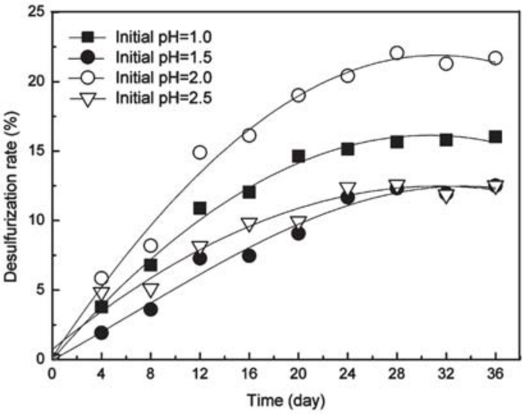

Fig. 4. Desulfurization rates throughout the bioleaching by a strain of extreme thermophilic bacteria

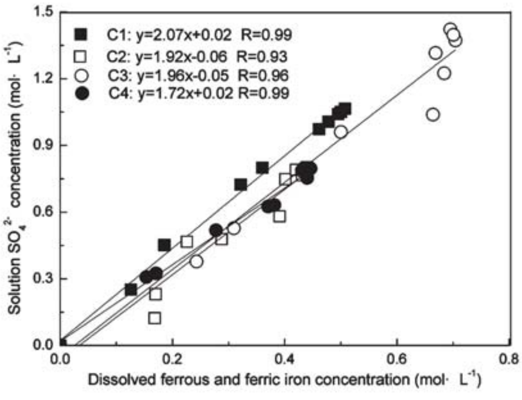

Fig. 5. Linear fitting of productive SO_4^{2-}/TFe (total dissolved iron) molar concentrations in pregnant solutions

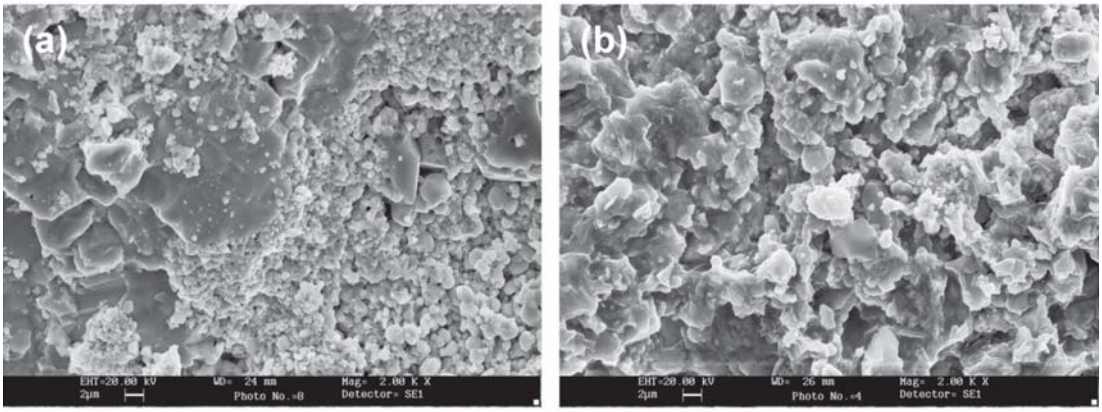

Fig. 6. Pyrite surface morphology (a) before and (b) after column bioleaching by extreme thermophilic bacteria: (a)before column bioleaching; (b)after column bioleaching

assisted leaching, jarosite is usually formed in the pH range of 1.35~3.0 in acidic media[18]. However, jarosite also forms under lower pH conditions with high $Fe_2(SO_4)_3$ concentrations at elevated temperatures. Xia and co-mates[10] reported that jarosite was detected during pyrite bioleaching by archaea *Acidianus manzaensis* at pH 0.85 and at 65°C. Similarly, the present work reveals that high iron concentration (39.2g/L) and temperature (70°C) promote the formation of jarosite at variable initial pH.

CONCLUSIONS

1. An extreme thermophile isolated from Tengchong hot spring optimally grows at pH 2.0 and at 70 °C in pure medium. This strain is spherical or oval-shaped, acidophilic and obligately autotrophic bacterium in iron-sulfur environment.
2. Inoculation of extreme thermophilic cultures leads to a rapid pyrite oxidization after a brief bacterial adaptation at pH 1.0~2.5 in column leaching. Initial pH affects the Fe(II) oxidization, redox potential and precipitation formation, therefore controls the iron leaching at elevated temperatures.
3. The highest pyrite desulfurization rate reaches 21.7% at initial pH 2.0 after 36 days leaching. Initial pH plays a key role in bacterial adaptation and activity, and further determines the oxidization of thiosulfate intermediates to sulfate. In the presence of high iron concentration (28.4g/L) at lower pH, Fe(III) ions are more susceptible to precipitate than sulfate ions.
4. During the process of pyrite biodesulfurization by extreme thermophilic bacteria, the smooth and integrate particle surface develops to a porous structure with typical bacterial corrosion pits. The jarosite and few elemental sulfur compose the hydroxysulfate precipitation in the matrix after bioleaching.

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