Precipitation of valuable metals from bioleaching solution by biogenic sulfides

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1. Introduction

While the world demand for all kinds of nonferrous metals is growing, the mineral industry is increasingly faced with the need to process low grade ores. Bacteria-assisted heap leaching and solvent extraction techniques used in low grade copper ore processing provide successful examples for low grade metal ore treatment (Aminian and Bazin, 2000; Navarro and Alguacil, 1999). A wide range of sulfide minerals (e.g. chalcolite, chalcopryite, pyrite, sphalerite and galena) can be dissolved in a bioleaching environment and a great deal of research work has been conducted (Laval-le et al., 2008; Plumb et al., 2002; Santos et al., 2006; Watling, 2006). However, there was little concern on how to handle efficiently with the bioleaching solution, which usually contains valuable metals at low concentration and a high level of impurity ions such as Mg\textsuperscript{2+}. It is difficult to separate and concentrate the valuable metals from these solutions by solvent extraction.

Sulfide precipitation has been demonstrated to be a successful method in processing laterite pressure leaching solution (Zhang and Cheng, 2007), which contains a high level of impurity ions. However, the high cost and safety problems in the process of producing H\textsubscript{2}S make this method unsuitable to be applied to processing bioleaching solution.

Sulfate-reducing bacteria (SRB) are obligate anaerobes characterized by their ability to perform dissimilatory sulfate reduction with simultaneous oxidation of organic substrates (Postgate, 1984). The metabolism of SRB also generates alkalinity, which contributes to neutralizing the acidity of industrial wastewater (Luptakova and Kusnirovova, 2005). Sulfate-reducing bacteria can use simple organic compounds as electron donors and sulfate as the external electron acceptor, which is in turn reduced to sulfide. The following are the general reactions used to describe the sulfate reduction process:

\begin{equation}
\text{Organic matter} + \text{SO}_4^{2-} \rightarrow \text{H}_2\text{S}^- + \text{HCO}_3^{-}
\end{equation}

\begin{equation}
\text{Me}^{2+} + \text{HS}^- \rightarrow \text{MeS}^- + \text{H}^+ (\text{Me}^{2+} \text{--- the metal cation})
\end{equation}

This process is based on the ability of SRB to reduce sulfates to hydrogen sulfides, which can form sparingly soluble precipitates of divalent metals as metal sulfides.

In last 20 years, biogenic sulfide precipitation has mainly been applied to the bioremediation of acidic mine drainage (AMD), which is one of the most important environmental problems (Benedetto et al., 2005; Bhagat et al., 2004; Elliott et al., 1998; Garcia et al., 2001; Jong and Parry, 2003; Luptakova and Kusnirovova, 2005; Tsukamoto et al., 2004). The efficiency of biological treatment of sulfate-loaded wastewater depends strongly on the outcome of the competition for common substrates between sulfate reducers and other bacteria in an anaerobic bioreactor, a process not yet fully understood. It was reported that the COD/SO\textsubscript{4}\textsuperscript{2-} ratio and pH in the reactor determined the outcome of competition (McCartney and Oleszkiewicz, 1993; Okabe et al., 1995). On the other hand, the heavy metals ions in the wastewater are toxic to microorganisms including SRB, because of their ability to deactivate enzymes by reacting with their functional groups, to denature proteins, and to compete with essential cations (Mazidji et al., 1992).
The objective of this research was to examine the possibility of the metal precipitation by biogenic sulfides, and establish a new method for the bioleaching solution treatment based on this process. In this study, tests were developed to determine if the bacteria produced hydrogen sulfide that could be used for the sulfide precipitation of valuable metals from a bioleaching solution. At the start of this research, batch experiments were carried out to determine the suitable conditions for production of biogenic sulfides and the valuable metals precipitation respectively, then the continuous process was developed in which the biogenic sulfides were produced in the first reactor and the valuable metals precipitation by the biogenic sulfides were realized consequently in the second reactor.

2. Materials and methods

2.1. Microorganisms and culture media

The mixed culture of SRB used in the present study was obtained from the anaerobic sludge on the bed of a sewage channel in Beijing, China. The enrichment culture was obtained as follows: The cultures were seeded with 10% sludge containing SRB and incubated at 35 °C in a glass master culture bottle. The medium was sparged with nitrogen gas (99.999%, Beijing Gaisi Chemical Gas Center, China) to maintain anaerobic conditions before inoculating. Every week 20% of the volume of the culture in the bottle was replaced by fresh medium. After several months, a culture containing high density of bacteria of different morphological types was achieved.

Postgate's medium B was used to prepare the enrichment culture (Postgate, 1984). This medium contained (in g/L): K₂HPO₄ 0.5, NH₄Cl 1.0, CaSO₄ 1.0, FeSO₄·7H₂O 0.5, sodium lactate 3.5, MgSO₄·7H₂O 2.0, yeast extract (Oxoid Ltd., Basingstoke, England) 1.0, ascorbic acid 0.1, thioglycollic acid 0.1. Modified Postgate's B medium containing 0.8 g/L K₂SO₄ instead of FeSO₄·7H₂O was used for batch experiments. All reagents except yeast extract were purchased from Sinopharm Chemical Reagent Beijing Co., and all chemicals used in this work were analytical grade reagents (AR).

Ceramic Raschig rings provided suitable support surface for immobilization of active SRB because of the formation of biofilms; the concentration of active SRB present as biofilms is substantially greater than the concentration of SRB in suspension culture (Silva et al., 2006; Tabak and Govind, 2003). The density of cells was approximately 2 × 10⁸ cells/mL in all batch experiments.

2.2. Determination of suitable conditions for production of biogenic sulfides

The effects of COD/SO₄²⁻ ratio and pH on the production of biogenic sulfides were investigated in batch experiments. Sealed flasks (250 mL) containing 180 mL modified Postgate B medium were used in these experiments. Sealed flasks were seeded with 20 mL inoculum from the enrichment cultures described above in anaerobic conditions under the protection of purging nitrogen gas. The inoculated flasks were incubated at 35 °C under static conditions. All experiments were conducted in duplicate and the results expressed as the mean value.

2.3. Valuable metals precipitation by the biogenic sulfides

With regard to sulfide precipitation, the preliminary experiments were carried out in batch tests using a 1 L reversed tapered precipitator with model bioleaching solutions containing Mg²⁺, Fe³⁺, Ni²⁺ and Cu²⁺. The following recipe was based on the real bioleaching solution from Jinchuan nickel pyrite in Gansu Province, China. The solution was prepared by dissolving MgSO₄·7H₂O (Mg²⁺ at ca. 20 g/L), Fe₂(SO₄)₃·XH₂O (Fe³⁺ at ca. 5 g/L), NiSO₄·6H₂O (Ni²⁺ at ca. 2 g/L) and CuSO₄ (Cu²⁺ at ca. 0.5 g/L) in de-ionized water.

2.4. Successive bacterial production of the hydrogen sulfide and valuable metals precipitation by biogenic hydrogen sulfides

A schematic of the experimental apparatus is illustrated in Fig. 1. The system consisted of two components: (1) a sulfate reduction bioreactor to produce H₂S; (2) a metal sulfide precipitator to bring bioleaching solutions in contact with biogenic hydrogen sulfide. The two stages are described as follows.

Stage 1: Biological production of H₂S. In this stage an anaerobic fixed bed bioreactor (Ø 7 cm × 56 cm) fabricated from Plexiglas...
was using, having a total volume of about 2 L and a working volume of 1 L. This reactor was filled with inert ceramic Raschig rings as carriers (Ø 10 mm x 10 mm) for supporting the biofilm and the height of the fixed bed was 0.3 m. The temperature in the fixed bed bioreactor was controlled at 35 ± 1 °C and heating was provided by an electrical heating tape. The synthetic substrate was prepared daily and stored in a substrate reservoir, which was continuously injected into the reactor through a peristaltic pump (BT01-100, Baoding Longer Precision Pump Co., China).

Stage 2: Valuable metals precipitation by biogenic sulfur. This stage followed after sufficient amount of H2S was produced. The bioleaching solution was pumped into the metal precipitator with 1 L working volume by a peristaltic pump.

Modified Postgate’s B medium containing K2SO4 instead of FeS2. H2O was used for seed substrate; the pH of the medium was initially adjusted to 7 with 1 M NaOH solution. The sulfate concentration in the substrate was increased by 500 mg/L periodically. Whenever appropriate the sulfate loading rate was increased by increasing the influent sulfate concentration without changing the influent flow rate or the COD/SO42- ratio. Experimental runs were carried out in batch- and continuous-flow mode bioreactors. The fixed bed reactor was initially operated in a batch mode with complete recycle until a fixed film of bacteria was formed on the ceramic Raschig rings carriers. Once 80% reduction of sulfate was achieved, the operation was changed to a continuous-flow mode. The following parameters were routinely monitored during the experiment: pH, oxidation reduction potential (ORP), total sulfide (TS) and sulfate concentration.

2.5. Analytical methods

A glass pH electrode (type E-201-C, Cany Precision Instruments Co., Shanghai, China) was used to measure pH and a Pt electrode (type 213, Shanghai Rousui Technology Co., China) combined with a reference saturated calomel electrode (the potential is 0.241 V SHE; type 232, Shanghai Rousui Technology Co., China) was used to measure the ORP. The total sulfide concentration was measured immediately after sampling using the iodometric method (Wei, 2002). Sulfate was measured according to the turbidimetric method (Kolmert et al., 2000) using a UV2000 spectrophotometer (Lab-Tech Co., USA), the absorbance of the sample was measured at a wavelength of 420 nm. The cell density was determined by direct counting with a Petroff–Hausser counting chamber under a microscope. Bacteria were viewed using an Olympus CX31 microscope. The dissolved metal concentrations were determined by inductively coupled argon plasma (ICP) emission spectroscopy (Perkin–Elmer Optima 5300DV). The precipitates were oven-dried in a hot air oven (DF206, Beijing Second Medical Facility Factory, China) at 80 °C for 8 h. The samples were carbon-coated first, and then analyzed by scanning electron microscopy (SEM) (Hitachi S-3500N) and energy spectrum (ES) (Oxford INCA).

3. Results and discussion

3.1. Effect of COD/SO42- ratio and pH on production of biogenic sulfides

The COD/SO42- ratio in the feed was an important parameter related to electron flow in anaerobic fermentation. The COD/SO42- ratio in the feed varied in the range of 0.5–9.0, while the COD loading rate was increased by increasing the feed lactate and yeast extract concentration without changing the sulfate concentration (1925 mg/L). The generation rate of biogenic sulfide was estimated by measuring sulfate removal rate. A higher removal efficiency of sulfate indicated the higher generation rate of sulfides and higher activity of SRB, while decreased sulfate removal efficiency implied decreased activities of SRB or their escape from the flasks. Variations of sulfate concentration at different COD/SO42- ratios with time during the batch culture are shown in Fig. 2.

From data presented in Fig. 2, it can be observed that while COD concentration increased from 963 mg/L to 17330 mg/L with the same concentration of SO42-, sulfate removal rate increased and strong sulfate-reducing activity was achieved under the initial COD/SO42- ratio of 3.0. This indicated that the higher COD concentration had a positive effect on the sulfate-reducing efficiency, but sulfate removal efficiency was more sensitive to the initial COD/SO42- ratio. This could easily be justified using the Monod equation, a higher concentration of COD provided sufficient organic carbon source for new SRB cells anabolism. On the other hand, the experimental data showed that a lower or higher COD/SO42- ratio might result in low sulfate removal efficiency in the reactor. Low sulfate reduction might be attributed to the inhibition of the anaerobic process due to lack of sufficient SRB (Mohanty et al., 2005). Some reports have suggested that at higher organic loading rates and in sulfate limiting conditions, methane producing bacteria (MPB) might dominate in the competition with SRB (Bhattacharya et al., 1996; Choi and Rim, 1991). And it appears that increasing COD concentration or COD/SO42- ratio increased the percentage electron flow by MPB (Isa et al., 1986), which had a little negative effect on sulfate reduction. Competitive interactions between these two groups of bacteria have been demonstrated by considering COD/SO42- ratios. Besides, the absolute value of concentration of sulfate is also very important. At the COD/SO42- ratio of 9, perhaps SRB competed substrate with other microbes such as acetogenesis or MPB due to surplus carbon source. Further research is needed to elucidate the effect of higher COD/SO42- ratio on SRB.

The pH value is a key factor affecting sulfate removal rate. It has been reported that sulfate reduction was usually inhibited at pH values lower than 6 or higher than 9 (Widdel, 1988). At low pH, the production of free H2S as a strong inhibitor of SRB is facilitated by the equation (H2S ↔ H+ + HS−). The pH values in this study ranged from 3 to 10.

In most flasks, the pH decreased immediately (Fig. 3a), due to acid-generating conversion after inoculation at certain extent and a possible mixing effect. The metabolic reaction of SRB fed with lactate as their organic carbon source, is described below:

\[2CH_3CH(OH)COO^- + SO_4^{2-} \rightarrow 2CH_3COO^- + 2HCO_3^- + HS^- + H^+ (\Delta G = -159.6 \text{ kJ/reaction})\]  

which indicates that 96 mg/L (1 mol) of sulfate removed leads to generation of 122 mg/L (2 mol) of alkaline species. After the initial
sharp decrease, the pH values ranged between 7.0 and 7.5 when the initial pH was between 6 and 9. These results indicated that the influence of initial pH level (between 6 and 9) had little or nothing to do with the ultimate pH for the system can adjust the pH by itself.

From Fig. 3b, it was found that the optimum initial pH of the mixed culture of SRB grown on ceramic Raschig rings and at a COD/SO$_2$ ratio of 3 was pH 7. Although the optimum initial pH of growth for this type of microorganisms is about 7, it was evident from the data that the mixed cultures tested were adapted to an initial pH of 5 without problems. However, bacteria grew with some difficulty on a moderately acidic medium (pH 3 and 4) and although a tendency for an increase in pH was observed, which indicates SRB activity, the sulfate removal efficiency was only about 33% in such excessively acidic medium. This growth difficulty in acidic medium has been mentioned often in the literature about 33% in such excessively acidic medium. This growth difficulty in acidic medium has been mentioned often in the literature and it is a controversial aspect in the possible treatment of AMD solutions, precipitation tests were carried out at various pH values under certain conditions, with pH of biodecoloring solution adjusted by means of adding solid CaCO$_3$. The results presented in Table 1 show that metal (Cu, Ni and Fe) removal efficiencies were generally high, while magnesium remained in the aqueous phase. The data in Table 1 indicated that the initial pH of the biodecoloring solution had no influence on valuable metal removal when biogenic H$_2$S was enough for metal sulfide precipitation.

The pH in the precipitator had a significant influence on the selective recovery of Cu, Ni and Fe sulfide concentrates from biocatalytic solutions, precipitation tests were carried out at various pH values. These results indicated that the influence of initial pH level (between 6 and 9) had little or nothing to do with the ultimate pH for the system can adjust the pH by itself.

**3.3. Metal precipitation by the biogenic sulfides**

In essence, the metal sulfide precipitate acted as a barrier that prevented the access of the electron donor–acceptor pair to the active bacterial site or enzyme that catalyzed the sulfate reduction. It has been reported that the effect of biogenic metal sulfides on SRB is not toxic (not causing mortality) but inhibitory in nature (Utgi-car et al., 2002). The reduction of H$_2$SO$_4$ to H$_2$S requires 8 electrons and this is described by the following stoichiometry (Herrera et al., 1997):

$$\text{SO}_4^{2-} + 4\text{H}_2 + 2\text{H}^+ \rightarrow \text{H}_2\text{S} + 4\text{H}_2\text{O}$$ (4)

The precipitation of metal sulfides in an organic substrate improves biocatalysis of solution quality by decreasing the mineral acidity without causing a parallel increase in proton acidity. Protons released by H$_2$S dissociation are neutralized by an equal release of HCO$_3$ during sulfate reduction. Apart from the operation conditions, the efficiency of metal precipitation with biogenic H$_2$S depends on the type of reactor, i.e., on the mass transfer in the precipitation (Foucher et al., 2001). The extraction processes were studied by batch experiments in order to determine the main effects and interactions of the valuable metal sulfide precipitation factors.

The pH in the precipitator had a significant influence on the metals removal since the rate of H$_2$S dissolution is faster at high pH levels. In order to determine the best pH values for the selective precipitation of metal sulfides precipitation. As shown in Table 2, the order of the removal rate of metals was: Cu > Fe > Ni > Mg. Copper removal was 100% in two experiments, while Fe removal was 62.67–100% and Ni removal was 46.4–100%. A light brown precipitate formed immediately in the precipitator when biocatalytic solutions were first in contact with the effluent hydrogen sulfide-containing solution. When the total sulfide was insufficient (420 ppm in Table 2), total metal sulfides precipitation were not achieved, whereas the presence of excess sulfide can lead to the formation of aqueous polysulfide complexes, which consume the sulfide reagent and decrease the metal removal efficiency (Lewis and van Hille, 2006).

The SEM picture of a precipitate sample from model biocatalytic solutions after sulfide precipitation is presented in Fig. 4. Some attachments adhered to the precipitate surface (the light clumps

![Fig. 3. Variations of pH and sulfate concentration at different initial pH values with time during the batch culture.](image-url)
in the photo), and this was probably the result of nucleation. The light phase was not markedly present in the micrograph of the final sample. From the ES pattern (Fig. 5), it was found that peaks of Fe, Ni, Cu and Mg were registered in the composition of precipitates, and these peaks were related with the representative components of the model bioleaching solution, which further confirmed that Fe, Ni and Cu were precipitated in the form of metal sulfides. In addition, the analysis through ES has shown that the light phase (light-coloured field in the micrograph) was mostly composed of sulfur, Fe and Ni, which indicates that precipitation took place mainly in the light phase. Therefore, it is clear that the formation of preferential precipitation sites was occurring, since SRB presumably create a suitable H2S-rich microenvironment for metal sulfide precipitation. In addition to precipitate as sulfide, heavy metals might also have been removed through sorption on the biomass. In fact, the presence of SRB cells has previously been shown to facilitate metal precipitation (Azabou et al., 2007).

Theoretically, increasing temperature can promote the reaction rate, and result in metal precipitation occurring more quickly. Therefore, the effect of temperature on the metal sulfide precipitation was investigated, and the results are presented in Table 3. It can be seen that there was no influence on Cu removal, while Ni removal efficiency increased with increasing temperature, Fe removal efficiency fluctuated with temperature, and at higher temperature the removal efficiency of Ni was higher than Fe. From the results, it also can be observed that a small quantity of Mg2+ precipitated at the higher temperatures, indicating that Mg2+ precipitated partly as insoluble bicarbonate or carbonate precipitates, which lead to the perplexing consequence disadvantageous to separating metals from Mg2+. At lower temperature, the formation of loose, spongiform flocs occurred, which would make the separation of precipitates from liquid more difficult. While at higher temperature, no loose, spongiform flocs forming, that indicated that the speed of precipitation was quicker and the size of sulfide precipitates was larger. Besides, solubility product constants of metal sulfides increases with temperature also lead to sulfide precipitation occurring quicker. As mentioned above, increasing temperature properly had a positive effect on the precipitation of valuable metals. According to Table 3, the best metal removal efficiency was obtained when the temperature was 60 °C.

Our experimental results showed that the success of selective precipitation of metals as sulfide solid depended strongly on the quantity of H2S from biological production. The suitable conditions determined from precipitation experiments were: pH of mixed SRB culture of around 7.30, but the most adequate pH value for precipitation and separation was 6.30, the temperature was 60 °C, the volume ratio of mixed SRB culture and synthetic bioleaching solution was 9:1 (450 mL:50 mL), and the concentration of TS in the bioleaching liquor was >500 mg/L. Under those conditions, ferric iron, copper and nickel precipitated immediately. Only two minutes after the mixing occurred, the concentrations of iron, copper and nickel in liquor were found respectively to be lower than their detection limits, 0.09 mg/L and 0.57 mg/L, whereas the magnesium concentration was almost unchanged. The results suggested that magnesium can be successfully separated from Fe, Ni and Cu, and more than 99% valuable metals can be recovered.

3.4. Continuous operation of bacterial hydrogen sulfide production and valuable metals precipitation

Fig. 6 summarizes the overall reactor performance during 267 days of operation. For continuous treatment the ceramic Raschig rings were pre-inoculated in batch experiments. Fig. 6a shows the sulfate concentrations. Initial sulfate concentrations ranged from 1926 to 3926 mg/L, whereas final sulfate concentrations ranged from 52 to 660 mg/L. Our batch experiments gave a similar result on sulfate removal rate. After 28 days of batch operation, the continuous treatment was started at a hydraulic residence time of 24 h. After reaching a constant effluent concentration, the throughput was increased gradually, giving rise to an increase in sulfate reduction within a period of about 7 months. When 1926 mg/L sulfate was added, 87% was removed, with approximately 215 mg/L sulfate in the reactor effluent. In contrast, when 2426 mg/L sulfate was added to the reactor influent, a greater proportion was reduced with an effluent concentration of 52 mg/L.

By comparing the sulfate removal rate with the pH and ORP data in the SRB column, it was found that fluctuations in sulfate concentration at different levels of the bed were related closely to the pH and ORP. For example, large changes in sulfate concentration were observed during the period from the 25th day to the 75th and similar fluctuations also occurred in either pH or ORP. The sulfate removal rate data implied that bacterial activity in the fixed bed reactor was higher near the top of the bed, where the SRB were well established. The pH and ORP data indicated that the environment at the top was ideal for their growth and activity, which accounts for the observed increase in bacterial activity here. If the SRB have access to a porous surface, the sulfate reduction rate is much higher in comparison to suspended microorganisms. Immobilization is obviously advantageous to SRB, perhaps due to a protective effect of the formed SRB biofilm.

Table 3

<table>
<thead>
<tr>
<th>Removal rate%</th>
<th>Temperature (°C)</th>
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<tbody>
<tr>
<td></td>
<td>Room temperature</td>
</tr>
<tr>
<td>Fe</td>
<td>85</td>
</tr>
<tr>
<td>Ni</td>
<td>95</td>
</tr>
<tr>
<td>Cu</td>
<td>100</td>
</tr>
<tr>
<td>Mg</td>
<td>0</td>
</tr>
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Note: SRB 45 mL, pH 7.22, [TS] 765 mg/L; bioleaching solution 5 mL.

Fig. 4. The SEM picture of the speckled area on surface of precipitate (300×).

Fig. 5. ES qualitative analysis of the surface of precipitate.
4. Conclusions

The following conclusions can be drawn from this study:

(1) The results of batch experiments showed that both COD/\(\text{SO}_4^{2-}\) ratio and initial pH value were important for influencing the reducing capability of SRB. A high removal rate of \(\text{SO}_4^{2-}\) was achieved when the initial COD/\(\text{SO}_4^{2-}\) ratio was around 3.0 and the initial pH value was around 7.0.

(2) The suitable conditions determined from precipitation experiments were: a pH value of the mixed SRB culture of 7.3, the most adequate pH value for precipitation and separation was 6.3, the temperature was 60 °C, the ratio of mixed SRB culture and synthetic bioleaching solution was 9:1 (450 mL: 50 mL), and the concentration of TS in bioleaching liquor was >500 mg/L.

(3) The mixed culture of SRB effectively separated valuable metals from bioleaching solutions by sulfide precipitation. The successive running of the hydrogen sulfide bacterial production and the valuable metals precipitation by biogenic sulfides, i.e. the application of two reactors, allowed faster valuable metals recovery, as well as the possibility of selective metals precipitation in the form of sulfides.

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