Hydrometallurgy 95 (2009) 127–134

Contents lists available at ScienceDirect

Hydrometallurgy

journal homepage: www.elsevier.com/locate/hydromet

Influence of Mg^{2+} on the growth and activity of sulfate reducing bacteria

Jun-ya Cao [a], Guang-ji Zhang [a,b,*], Zai-sha Mao [a], Zhao-heng Fang [a], Chao Yang [a,b], Bao-ling Han [b]

[a] Key Laboratory of Green Process and Engineering, Institute of Processing Engineering, Chinese Academy of Sciences, Beijing 100190, China
[b] National Key Laboratory of Biochemical Engineering, Institute of Processing Engineering, Chinese Academy of Sciences, Beijing 100190, China

ARTICLE INFO

Article history:
Received 22 August 2007
Received in revised form 29 January 2008
Accepted 10 May 2008
Available online 17 May 2008

Keywords:
Sulfate reducing bacteria
Magnesium
Ionic concentration
Salinity
Precipitation

ABSTRACT

Bioleaching liquors usually contain low concentration valuable metals and a major impurity (magnesium or calcium), and therefore it is a heavy burden and involves a complicated process to separate and enrich valuable metals from these liquors. An alternative process of selective precipitation of metals using H_2S produced by sulfate reducing bacteria (SRB) is put forward. In this paper, sealed flasks were used to study the influence of Mg^{2+} in the form of $MgCl_2{\cdot}6H_2O$ and $MgSO_4{\cdot}7H_2O$ on the growth and activity of SRB, and the sulfide precipitation of bioleaching liquors was also studied. The experimental results show that Mg^{2+} ion concentration from $MgCl_2{\cdot}6H_2O$ or $MgSO_4{\cdot}7H_2O$ is a significant and favorable factor for SRB growth and activity. It is also demonstrated that enhanced activation of SRB by adding NaCl in liquor and the SRB have a high tolerance to salinity from NaCl. Adding $MgSO_4{\cdot}7H_2O$ provides a predominant condition for SRB growth, due to providing higher concentration of sulfate. The sulfide precipitation and separation of magnesium with H_2S produced by SRB is found to be mainly dependent on the pH value and the H_2S concentration. SRB culture solution can effectively precipitate Cu^{2+}, Ni^{2+} and Fe^{3+} from synthetic leaching liquor in 2 min, while Mg^{2+} ions remain in the liquid phase.

1. Introduction

Since the 1960s, the role of bacteria in leaching was known and the widespread use of heap and in situ leaching for extracting copper has been reported (Brierley and Brierley, 2001; Ehrlich, 2001; Habashi, 2005). The biological recovery of metals from low-grade ores and mineral concentrates has been developed into a successful and expanding area of biotechnology (Bosecker, 1997; Mousavi et al., 2007; Qiu et al., 2005). The bioleaching of sulfide ores received increasing attention due to the advantages of low consumption of energy and environment friendliness (Shi and Fang, 2005). However, the applications of heap bioleaching are at present mainly practiced with the extraction of copper and uranium (Kodali et al., 2004). More research is still required before the final goal of extending bioleaching to more kinds of ores is fulfilled. The increasing demand for nickel and cobalt has led to thorough exploration for ore sources. Besides, the extraction of valuable metals from low-grade sulfide ores by bioleaching is becoming more and more promising. One of the key technical problems is how to process the bioleaching liquor.

Bioleaching liquors contain valuable metals at low concentration and a high level of impurity ions, and the separation of them is difficult due to complex composition and high concentration of impurity. The processing technologies to separate valuable metals from major impurities include direct solvent extraction, intermediate precipitation and ion exchange accordingly (Annamalai and Murr, 1979; Donegan, 2006; Karavasteva, 1998; Muresan et al., 1996; Pinto and Martins, 2001). Among them precipitation of metal as sulfide has gained prominence in recent decades, which is recognized as an effective and important way to extract and enrich low level valuable metals. It was reported that nickel and cobalt successfully recovered from laterite leaching liquors by sulfide precipitation (Committee, 1999). Since H_2S is a very toxic gas, producing, transporting and using of H_2S are relatively expensive, because the serious issues for environmental safety lead to high operating cost. Therefore, the precipitation of metals with biologically produced H_2S by sulfate reducing bacteria (SRB) was suggested as an alternative process (Foucher et al., 2001). SRB are anaerobes characterized by their ability to perform dissimilatory sulfate reduction with the simultaneous oxidation of the organic substrates (Postgate, 1984a,b). Anaerobic treatment processes have been applied to several industrial waste treatment situations since the 1990s (Johnson, 2006). This process is based on the following two fundamental reasons (Garcia et al., 2001). Firstly, SRB has the capacity to reduce sulfate to sulfide, which then reacts with certain metals to form insoluble precipitates. Secondly, the system acidity is reduced by their own action of sulfate reduction and by the carbon metabolism of the bacteria. In the last 20 years, research emphasis was mainly focused on the bioremediation of acidic mine drainage (AMD), since it is one of the most important environmental problems (Elliott et al., 1998; Jong and Parry, 2003; Luptakova and Kusnierova, 2005; Tsukamoto et al., 2004).

However, none of these studies attempted to use this biological process to treat leaching liquors, which contained low level valuable

* Corresponding author. Tel.: +86 1062554558; fax: +86 1062561822.
E-mail address: gjzhang@home.ipe.ac.cn (G.-J. Zhang).

0304-386X/$ – see front matter © 2008 Elsevier B.V. All right reserved.
doi:10.1016/j.hydromet.2008.05.006

metals and high level alkali-earth metals such as magnesium. Bioleaching liquor is strongly acidic with pH value about 2.0, and the concentrations of heavy metals are higher than that in AMD. Only limited literature data are available, showing the influence of ionic concentration on the growth and activity of SRB, which is closely related to the efficient biological treatment of bioleaching liquor. Blight and Ralph (2004) reported that ionic strength was a significant variable to be considered when quantitatively modeling the growth of chemolithotrophic cells (Blight and Ralph, 2004). It is necessary, therefore, to examine the role of ionic concentration in SRB growth.

The long term objective of the study conducted in our laboratory is to develop the biological treatment process of bioleaching liquor containing low concentration sulfate and valuable heavy metals. This work is part of the objective and aimed (1) to determine the influence of Mg^{2+} on the reducing capability of SRB, (2) to separate metals as sulfide precipitations from solution containing magnesium. It will ensure the highest sulfide production under consistent and steady state conditions, which will provide the most suitable environments for metal precipitation. The reason of choosing Mg^{2+} is that bioleaching liquor from low-grade nickel ores contains high levels of magnesium (about 20 g/L). Mg^{2+} beyond certain concentration maybe become harmful to SRB and will affect the precipitation and separation of metal sulfides, thereby deleteriously affecting the biotreatment efficiency. Batch tests were carried out in flasks to study the influence of Mg^{2+} in the form of $MgCl_2{\cdot}6H_2O$ and $MgSO_4{\cdot}7H_2O$ on the reducing capability of SRB. The influence of salinity on the growth of SRB is also addressed.

2. Materials and methods

2.1. Microorganisms

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. Postgate's B medium was used for preparation of active SRB cultures (Postgate, 1984a,b). The enrichment culture was developed as follows: The sludge was seeded in a 2-L glass master culture bottle. The cultures were seeded with 10% sludge and incubated at 35 °C. The drain-and-fill schedule for the glass master culture bottle involved weekly replacement of 20% of the volume by fresh Postgate's B medium. The growth of SRB was confirmed by the formation of black precipitates, in addition the rotten-egg smell provoked by the hydrogen sulfide was obvious.

2.2. Culture media

Postgate's B medium (concentration in g/L): K_2HPO_4 0.5, NH_4Cl 1.0, $CaSO_4$ 1.0, $FeSO_4{\cdot}7H_2O$ 0.5, sodium lactate 3.5, $MgSO_4{\cdot}7H_2O$ 2.0, yeast extract 1.0, ascorbic acid 0.1, thioglycollic acid 0.1. Modified Postgate's B medium containing 0.8 g/L K_2SO_4 instead of $FeSO_4{\cdot}7H_2O$ was used for batch experiments. pH of the medium was initially adjusted to 7.0 with 1 M NaOH solution; the medium was sparged with nitrogen gas sufficiently to maintain anaerobic conditions.

2.3. Flask experiments

Additional $MgCl_2{\cdot}6H_2O$ was added into modified Postgate's B medium for adjusting Mg^{2+} to desired concentration, two sets of five concentration levels of Mg^{2+} were applied: in one set Mg^{2+} at ca. 10, 20, 50, 100 g/L, and in the other set Mg^{2+} at ca. 2, 5, 6, 8 g/L. Modified Postgate's B medium without $MgCl_2{\cdot}6H_2O$ was designated as control. The ratio of chemical oxygen demand to sulfate (COD/SO_4^{2-}) was initially maintained at 3.0 in all batch experiments, as determined previously (Cao et al., 2007). All experiments were conducted in triplicate and the averages were reported. 250 mL sealed flasks were used in batch experiments. In a previous study, we demonstrated that synthetic sponge (polyurethane foam) was a good immobilization carrier for supporting the biofilm growth (Cao et al., 2007). In the present study, synthetic sponge 30 mm high and 80 mm in diameter was used as immobilization carrier. The flasks experiments were seeded with 20 mL of the mixed SRB culture into 180 mL modified Postgate's B medium under the anaerobic condition by purging the flask with nitrogen gas. The inoculated flasks were incubated at 35 °C. Samples were withdrawn at certain intervals for the analysis of total sulfide (TS) and sulfate concentration, and an equal volume of medium was added to make up the total volume of the culture. The values of pH and oxidation reduction potential (ORP) of the solution were also monitored using the respective probes.

The influence of salinity on the growth of SRB was investigated to test if Cl^- has a significant effect on SRB. NaCl was dissolved in modified Postgate's B medium at three concentration levels, and the content of Cl^- was equal with Cl^- when testing with $MgCl_2{\cdot}6H_2O$ (Mg^{2+} at ca. 5, 8, 10 g/L). In this experiment, modified Postgate's B medium was designated as control, and the experimental procedures were the same as described above.

For the batch experiments using $MgSO_4{\cdot}7H_2O$ as the source of Mg^{2+}, no other sulfate was added. Five concentration levels of Mg^{2+} were studies: Mg^{2+} at ca. 1, 2, 5, 10, 20 g/L. As mentioned, COD/SO_4^{2-} ratio was initially maintained at 3.0, and modified Postgate's B medium was designated as control. The experimental procedures were the same as described above.

2.4. Sulfide precipitation

With regard to sulfide precipitation, the preliminary experiments were carried out in batch tests using a 1-L reversed tapered precipitator. The simulating bioleaching liquor contained $MgSO_4{\cdot}7H_2O$ (Mg^{2+} at ca. 20 g/L), $Fe_2(SO_4)_3{\cdot}XH_2O$ (Fe^{3+} at ca. 5 g/L), $NiSO_4{\cdot}6H_2O$ (Ni^{2+} at ca. 2 g/L), $CuSO_4$ (Cu^{2+} at ca. 0.5 g/L). Several factors were tested to optimize the sulfide precipitation of bioleaching liquor, including pH value, volume ratio of mixed SRB culture and synthetic bioleaching solution and the concentration of TS. The precipitator was initially inoculated with different volumes of mixed culture of SRB and then filled with the bioleaching liquor of the desired metal concentration.

2.5. Analytical methods

pH and ORP can be seen as indicators and controllers of the flask culture performance. A glass pH-electrode combined with a Pt electrode and a reference saturated calomel electrode (the potential is 0.241 V SHE) were used to measure pH and ORP respectively. A pH-meter (model PHS-3E, Lei Ci Company, Shanghai) was used, which was calibrated with pH 6.8 and 4.0 buffers. The total sulfide concentration was measured immediately after sampling using the iodometric method (Wei, 2002). Prior to sulfate analysis, 1 mL of 0.04 N $ZnCl_2$ solution was added to the sample to remove the sulfide through precipitation. Sulfate was then measured according to the turbidimetric method using a spectrophotometer (LabTech Co., USA), the absorbance of the sample was measured at wavelength of 420 nm (Kolmert et al., 2000). COD concentration was determined by the dichromate method. The cell density was determined by direct counting with a Petroff–Hausser counting chamber under a microscope. The dissolved metal concentrations were determined by inductively coupled argon plasma (ICP) emission spectroscopy (Perkin-Elmer Optima 5300DV). Samples were withdrawn, filtered for analysis of organic compounds at the end of batch experiments. An Agilent 1100 HPLC fitted with a ZORBAX Extented-C_{18} column and a VWD detector was used for this purpose. The mobile phase was acetonitrile and 0.02 M KH_2PO_4 (pH 2.8), flow rate and the ratio of organic solvent were controlled by a gradient elution program with a quaternary pump, the column temperature was set at 30 °C and wavelength of 215 nm was used for detection (Liu et al., 2006). The precipitate gained under the optimized conditions was dried at 80 °C for 8 h. The precipitate samples were carbon-coated first, and then analyzed by SEM (with Hitachi S-3500N) and ES (with Oxford INCA).