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

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**A B S T R A C T**

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\(_2\)S 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\(_2\)-6H\(_2\)O and MgSO\(_4\)-7H\(_2\)O 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\(_2\)-6H\(_2\)O or MgSO\(_4\)-7H\(_2\)O 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\(_4\)-7H\(_2\)O provides a predominant condition for SRB growth, due to providing higher concentration of sulfate. The sulfide precipitation and separation of magnesium with H\(_2\)S produced by SRB is found to be mainly dependent on the pH value and the H\(_2\)S 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.

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**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 limited 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 Mur, 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 sulfi des precipitation (Committee, 1999). Since H\(_2\)S is a very toxic gas, producing, transporting and using of H\(_2\)S are relatively expensive, because the serious issues for environmental safety lead to high operating cost. Therefore, the precipitation of metals with biologically produced H\(_2\)S 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 (García 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 Kusnirova, 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...
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 containing low concentration sulfate and valuable heavy metals. This therefore, to examine the role of ionic concentration in SRB growth.

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, 1984b,a). 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\(_2\)HPO\(_4\) 0.5, NH\(_4\)Cl 1.0, CaSO\(_4\) 1.0, FeSO\(_4\)-7H\(_2\)O 0.5, sodium lactate 3.5, MgSO\(_4\)-7H\(_2\)O 2.0, yeast extract 1.0, ascorbic acid 0.1, thioglycolic acid 0.1. Modified Postgate's B medium containing 0.8 g/L K\(_2\)SO\(_4\) instead of FeSO\(_4\)-7H\(_2\)O 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\(_2\)-6H\(_2\)O 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\(_2\)-6H\(_2\)O 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 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\(_2\)-6H\(_2\)O (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\(_4\)-7H\(_2\)O 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\(_4\)-7H\(_2\)O (Mg\(^{2+}\) at ca. 20 g/L), Fe\(_2\)(SO\(_4\))\(_3\)-XH\(_2\)O (Fe\(^{3+}\) at ca. 5 g/L), NiSO\(_4\)-6H\(_2\)O (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–Hauser 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-C18 column and a VWD detector was used for this purpose. The mobile phase was acetonitrile and 0.02 M KH\(_2\)PO\(_4\) (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).
All chemicals used in this study were analytical grade reagents (AR) unless otherwise stated; all solutions were prepared with deionized water. All plastic ware and glassware were thoroughly cleaned by soaking in 10% HCl for 24 h, and oven dried before use.

3. Results and discussion

3.1. Tolerance of SRB to MgCl₂

A culture of SRB has been previously cultured more than six months, and a high density culture of bacteria with different morphological types was achieved. The density of cells was approximately $2 \times 10^8$ cells/mL in all bioleaching experiments. In the present work, the tolerance of SRB to Mg²⁺ was tested by adding MgCl₂·6H₂O to the culture solutions. The experimental results are summarized in Figs. 1 and 2.

3.1.1. Tolerance experiment with high concentration MgCl₂

Fig. 1(a) presents the pH value versus time for high concentration MgCl₂ media. It should be noticed that the pH values always ranged...
between 6.0 and 7.5, and the ending pH increased with magnesium concentration. Interestingly, the trend of pH was similar between 50 g/L and 100 g/L, which indicated the influence of Mg\(^{2+}\) level (more than 50 g/L) had little or nothing to do with pH. pH increased gradually when the initial concentration of Mg\(^{2+}\) was more than 20 g/L in the first day, and pH decreased gradually when the initial concentration of Mg\(^{2+}\) was less than 20 g/L. These phenomena might due to acid-generating conversion after inoculation. The metabolic reaction of SRB, which fed with lactate as its organic carbon source, is described below:

\[
2\text{CH}_3\text{CHOHCOO}^- + \text{SO}_4^{2-} \rightarrow 2\text{CH}_3\text{COO}^- + 2\text{HCO}_3^- + \text{HS}^- + \text{H}^+ (\Delta G = 159.6 \text{kJ/reaction})
\]

It was found that 1 mol of sulfate was removed while 2 mol of alkaline species generated. This reaction probably took place faster in the flasks containing higher concentration of Mg\(^{2+}\) (Fig. 1(c)), which resulted in higher pH when more Mg\(^{2+}\) was added in the solution. Variations of ORP at different initial magnesium concentrations during the batch culture are shown in Fig. 1(b). It can be found that the ORP value always in the range of −120 mV and −360 mV, in spite of the almost same initial values (around −190 mV), which provided a proper condition for SRB growth. As indicated in Fig. 1(b), the ORP decreased rapidly in the first day, probably caused by new generated sulfides.

Fig. 1(c) shows typical results of sulfate reduction, which was generally promoted by Mg\(^{2+}\) addition. The sulfate consumption began soon after inoculation and its concentration decreased rapidly in the first day. This was probably due to enhanced biological activity of SRB as a result of increased concentration of Mg\(^{2+}\). However, after 1 day sulfate reducing activity in all tests increased slowly with time except the control in which the rate declined linearly roughly in 7 days. The same phenomena have been noticed by Oyekola and O’Flaherty, who reported a similar trend of fluctuating sulfate concentration in an anaerobic digestor treating propionate, butyrate and ethanol containing wastewater (O’Flaherty and Colleran, 1999; Oyekola and Pletschke, 2006). A possible reason for this phenomenon is that the reverse reaction of the sulfate activation resulting from the limitation in adenosine triphosphate (ATP) and accumulation of adenosine phosphosulphate (APS) occurred. ATP plays an important role in dissimilatory sulfate reduction, being the first enzyme involved in the activation of the inactive sulfate substrate (Pletschke et al., 2002). At the end of the test runs, there were 78%, 77%, 75%, 54%, 53% of sulfate removed respectively, and strong sulfate reducing activity is achieved in the system with Mg\(^{2+}\) at ca. 100 g/L, demonstrating that the SRB’s sulfate reducing activity was promoted strongly with high Mg\(^{2+}\). Similar to the case of the pH behavior, the removal rate of sulfate increased with magnesium concentration at the end of test run as indicated in Fig. 1. All these results indicated that the Mg\(^{2+}\) ions were not harmful to the SRB growth under this high concentration; the higher level of Mg\(^{2+}\) even stimulated the growth of SRB to some extent. This is considered to be due to better mass transfer condition or to improved diffusion of SO\(_4^{2-}\) ion into the microbe caused by increasing in Mg\(^{2+}\) level in the flasks, and further research is needed to elucidate the stimulatory effect of the rather high Mg\(^{2+}\) concentration (100 g/L) on SRB.

3.1.2. Tolerance experiment with low concentration MgCl\(_2\)

In addition to demonstrating the influence of Mg\(^{2+}\) on the growth and activity of SRB, a set of experiments with lower level concentration of Mg\(^{2+}\) was also conducted. As seen from Fig. 2(a), the variation trend of pH was very similar: pH decreased in the first 2 days, fluctuated gradually from 2 to 8 days, and fluctuated the last 4 days. pH was also maintained between 6.0 and 7.5 during operation. From Fig. 2(a) and (b), it can be seen that abrupt decreases in pH and ORP in the first 2 days and then fluctuated slightly. ORP was in the range of −100 mV to −400 mV, and increased with the initial magnesium concentration, which is similar with the former experiment. It should be noticed that the initial ORP was around −110 mV when adding MgCl\(_2\), which was higher than that in the former experiment. This may explain the lower removal rate of sulfate in this set of experiment (Fig. 2(c)).

In this experiment, Mg\(^{2+}\) level appeared to have a strong influence on the growth and activity of SRB, as indicated by the sulfate conversion rate and efficiency. The sulfate concentration in Fig. 2(c) reveals a general decreasing trend in all tests, indicating increasing rate of sulfate removal. Moreover, the highest sulfate reduction rate appeared when the initial concentration of Mg\(^{2+}\) was 2 g/L in the 10 day test and 71% of sulfate was removed. In other tests, only about 50% sulfate was removed at the end of the experiment, which indicated satisfactory overall performance of the systems. The order of sulfate removing rate was 2 g/L > control > 5 g/L > 6 g/L > 8 g/L.

**Fig. 3. Results of batch experiments with salinity.**
It was reported that the ionic strength of solution imposes an energy load on bacteria due to the osmotic gradient that exists between the interior and exterior of any cell (Blight and Ralph, 2004). Our experiment results above show that in the condition with higher level of MgCl₂·6H₂O, it was better for the growth and activity of SRB under the lower initial ORP.

3.1.3. Tolerance of SRB to Cl⁻

For comparison purpose and in an attempt to understand the influence of Cl⁻, experiments on tolerance of Cl⁻ were conducted. Four concentration levels of NaCl were tested with the content of Cl⁻ equal to Cl⁻ in MgCl₂·6H₂O tests (Mg²⁺ at ca. 5, 8, 10 g/L, namely Cl⁻ at ca. 14.8, 23.7, 29.6 g/L). Results obtained in three concentration levels are summarized in Fig. 3.

Comparing the pH between the samples in which MgCl₂·6H₂O (Fig. 2(a)) and NaCl (Fig. 3(a)) were added respectively, it can be found that the pH in the former fluctuated slightly to below 7.0, whereas the pH in the latter was further increased up to 7.6. The latter can provide lower ORP which was good to the growth of SRB. As shown in Fig. 3(c), in the first 10 days of the experiment, the sulfate removal was not affected by the addition of NaCl, sulfate in all systems was reduced almost at the same rate, which indicated that adding NaCl were stimulatory for SRB at the same extent during this period. In the last operational periods (days 10–
16), the salinity level appeared to affect the activity of SRB. This was expected since several species of SRB have been enriched and activated by adding NaCl. The highest sulfate reduction rate was obtained when the initial concentration of Cl$^-$ was 14.8 g/L, and 94% sulfate was removed, higher than that Mg$^{2+}$ added. Apparently, a successful sulfate reducing process could be obtained by adding NaCl. The higher sulfate removal rate suggested that SRB can be activated effectively. In addition, it shows that the biomass had a higher affinity for NaCl than for MgCl$_2$·6H$_2$O. As mentioned above, it was found that the SRB had higher tolerance of salinity, much more than the previously reported. It was reported that fresh-water strains were sensitive, with only small proportions of the population able to grow above about 1% NaCl and none above about 3% NaCl (Postgate, 1984a,b).

3.2. Tolerance of SRB to MgSO$_4$

To understand the influence of Mg$^{2+}$ deeply, comparative experiments were performed between MgCl$_2$·6H$_2$O and MgSO$_4$·7H$_2$O. Fig. 4 presents the experimental results with MgSO$_4$·7H$_2$O. As seen from Fig. 4(a), variation trend of pH were similar to the former experiments. Variation of pH is closely related to the concentration of biogenic H$_2$S, as shown in Fig. 4(c). The chemical equilibrium of the sulfide species is pH dependent. At pH 8 most of the total sulfide is in the form of HS$^-$, while at pH 6 most is H$_2$S. The results indicated the more MgSO$_4$·7H$_2$O contained in the system, the more free H$_2$S produced. It can be observed that the concentrations of TS were high due to abundant source of sulfate and high SRB activity.

From Fig. 4(b), it was seen that ORP hardly changed with 20 g/L MgSO$_4$·7H$_2$O. This is due to strong inhibition to the activity of SRB caused by high concentration of immediate biogenic H$_2$S. Hydrogen sulfide is toxic to SRB and can reduce the activity of SRB. It was reported that the hydrogen sulfide can lead to the decline of SRB activity at a low of 40 mg/L and a high of 124 mg/L (Kaksonen et al., 2004; Li et al., 1996). In this experiment, activity of SRB was found to decline when total sulfide concentration was around 1100 mg/L. ORP decreased with increasing concentration of MgSO$_4$·7H$_2$O in other systems. Previous report indicated that the SRB were not able to outcompete methane producing bacteria (MPB) for acetate, and SRB appear to be more sensitive than the MPB to the elevated TS concentrations (McCartney and Oleszkiewicz, 1991). This would also account for the observed tiny variation of ORP in the system containing 20 g/L Mg$^{2+}$ here.

Unlike in other reports, sulfate was removed quickly in 6 h after inoculation, and the sulfate reduction is correlated to COD oxidation, as indicated by the sharp drop in the first 6 h (Fig. 4(d) and (e)). Effective sulfate removal was observed in all systems. Comparison of sulfate removal data between adding MgCl$_2$·6H$_2$O and MgSO$_4$·7H$_2$O, it can be observed that MgSO$_4$·7H$_2$O was more advantageous over MgCl$_2$·6H$_2$O, probably due to the former contained higher sulfate and carbon source concentration so the activity of SRB would be stimulated by sulfate ions. It was assumed that an ionic substance such as either Na$_2$SO$_4$ or K$_2$SO$_4$ interferes with cell growth only through its contribution to ionic strength and not by any metabolic inhibition mechanism. In these experiments, the sulfate consumption was similar as evidenced in Fig. 4(d) and indicated that about 80% sulfate was removed at the end of a run in most flasks and the systems with MgSO$_4$·7H$_2$O added were slightly more effective in the removal of sulfate and COD than control. From Fig. 4(e), more than 65% of COD was removed in most systems, except the system with 1 g/L MgSO$_4$·7H$_2$O in which the removal efficiencies was much higher than our preliminary studies. Up to 86% of COD was removed in the flask with 20 g/L Mg$^{2+}$ at the end of a run. It was reported that the factors affecting the COD removal rate of high sulfate-containing wastewater were (1) the level of undissociated sulfide in liquid, (2) the COD/SO$_4^{2-}$ ratio of the influent, and (3) the volumetric loading rate of

<table>
<thead>
<tr>
<th>Initial [Mg$^{2+}$] (g/L)</th>
<th>1 g/L</th>
<th>2 g/L</th>
<th>5 g/L</th>
<th>10 g/L</th>
<th>20 g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Mg$^{2+}$] (g/L)</td>
<td>0.59</td>
<td>1.02</td>
<td>2.20</td>
<td>4.82</td>
<td>8.69</td>
</tr>
</tbody>
</table>

| Table 2 |
| Comparison of end-product distribution among different initial [MgSO$_4$·7H$_2$O] (mg/L) |

Table 1

<table>
<thead>
<tr>
<th>Mg$^{2+}$</th>
<th>Formic acid</th>
<th>Lactic acid</th>
<th>Acetic acid</th>
<th>Propionic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>27.5</td>
<td>331.8</td>
<td>–</td>
</tr>
<tr>
<td>1 g/L</td>
<td>–</td>
<td>83.1</td>
<td>620.2</td>
<td>86.2</td>
</tr>
<tr>
<td>2 g/L</td>
<td>–</td>
<td>331.3</td>
<td>219</td>
<td>222.2</td>
</tr>
<tr>
<td>5 g/L</td>
<td>1150</td>
<td>3560</td>
<td>2177</td>
<td>241.5</td>
</tr>
<tr>
<td>10 g/L</td>
<td>874</td>
<td>6174</td>
<td>2858</td>
<td>12,334</td>
</tr>
<tr>
<td>20 g/L</td>
<td>3457.5</td>
<td>24,625</td>
<td>1642.5</td>
<td>14,090</td>
</tr>
</tbody>
</table>

Fig. 5. The SEM picture of the speckled area on surface of precipitate (a) 300×; (b) 4000×.

Fig. 6. ES qualitative analysis of the surface of precipitate.
sulfate. Amongst them, the level of undissociated sulfide was regarded as the most influential (Yamaguchi et al., 1999). However, it was found that the sulfate concentration might be the most important factor to SRB activity in our study. This higher COD removal rate was probably caused by other microorganism such as MPB. As can be seen from Table 1, the main volatile fatty acids (VFA) increased with the concentration of Mg$^{2+}$, and VFA were acetate and lactic acids during the test period. This indicated that SRB can only incompletely degrade the carbon sources to acetate.

From the results of Table 2, it can be observed that the actual Mg$^{2+}$ concentration in the flasks was almost half of the initial concentration, indicating that loss of the metal ions had precipitated as insoluble bicarbonate or carbonate precipitates, which can be obtained in such a reaction:

$$\text{Organic matter(C, H, O)} + \text{SO}_4^{2-} \xrightarrow{\text{SRB}} \text{HS}^- + \text{HCO}_3^-$$

Moreover, sulfate reduction increased carbonate ion activity, and decreased the hydration energy of magnesium in water, promoting MgCO$_3$ formation in the microenvironment around the bacterial cells.

3.3. Sulfide precipitation from bioleaching solution

Except the operation conditions, the efficiency of metal precipitation with H$_2$S depends on the type of reactor, i.e. on the mass transfer in the precipitation (Foucher et al., 2001). Our experimental results show that the possibility of selective precipitation of metals as sulfide solid and separation with magnesium depending on the pH value in solution and the quantity of H$_2$S from biological production. In this study, the removal of ferric iron, copper and nickel was assessed.

The optimum conditions determined from precipitation experiments were: the pH value of mixed SRB culture was at 7.30, the most adequate pH value for precipitation and separation was 6.30, the volume 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 higher than 500 mg/L. Under those conditions, ferric iron, copper and nickel precipitated immediately, 2 min later the concentration of ferric iron, copper and nickel in liquors was found respectively to be lower than its detection limit, 0.09 mg/L and 0.57 mg/L, while the magnesium concentration was almost unchanged. The results suggested that magnesium can be successfully separated from Fe, Ni and Cu, and the recovery rates of valuable metals are more than 99%.

From the SEM photos and energy spectrum (ES) of a precipitate sample from sulfide precipitation (see Figs. 5 and 6), it was further confirmed that Fe, Ni and Cu were precipitated in the form of metal sulfides. The ES showed that this precipitation consisted of S, Fe, Ni and Cu (Fig. 6). Furthermore, it was noticed that the content of sulfur and ferric iron was not high in the light phase (light-colored regions in Fig. 5), suggesting that precipitation took place mainly in the dark regions. In addition to precipitate as sulfide, heavy metals might also have been removed through sorption on the biomass. Microbes have different defense strategies including complexing, extra-cellular precipitation, impermeability, or reduced transport of metals across cell membrane. In fact, the presence of SRB cells was shown to facilitate metal precipitation, and SRB have an additional, important role in facilitating metal precipitation (Azabou et al., 2007).

4. Conclusions

Based on the results obtained in this work, it can be concluded that:

(1) This paper shows that the effect of Mg$^{2+}$ on the growth and activity of SRB is highly concentration dependent. From the tests on tolerance of SRB to MgCl$_2$·6H$_2$O, the removal rate of sulfate increased with magnesium concentration at the end of the first test run. The system with high level Mg$^{2+}$ offers an advantage to the growth and activity of SRB, and strong sulfate reducing activity is achieved in the system with Mg$^{2+}$ at ca. 100 g/L.

(2) Adding NaCl can also make SRB more vital. The highest sulfate reduction rate was obtained when the initial concentration of Cl$^-$ was 14.8 g/L and 94% sulfate was removed, higher than that Mg$^{2+}$ added. The results show that SRB has higher tolerance to salinity, and is more sensitive to Mg$^{2+}$ rather than Cl$^-$. 

(3) Adding MgSO$_4$·7H$_2$O can provide more favorable conditions for growth of SRB, about 80% sulfate and more than 65% COD can be removed effectively in 2 days.

(4) The pH value and the quantity of H$_2$S produced by SRB are important factors for the precipitation of metal sulfides. Cu$^{2+}$, Ni$^{2+}$ and Fe$^{3+}$ ions in the synthetic bioleaching liquor are effectively precipitated as sulfides by SRB culture liquor in 2 min, while Mg$^{2+}$ ions remain in liquor.

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