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# Function of microorganism and reaction pathway for carrollite dissolution during bioleaching

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**Abstract:** The function of microorganism and dissolution reaction pathway of carrollite in the bioleaching process were investigated. The results showed that both indirect and contact mechanisms influenced the leaching process. The dissolution of carrollite was significantly accelerated when bacteria were adsorbed on the mineral surface, indicating that the contact mechanism significantly affected the dissolution of carrollite. During bioleaching, the sequence of oxidation state of the sulfur moiety of carrollite was as follows:  $S^{-2} \rightarrow S^0 \rightarrow S^{+4} \rightarrow S^{+6}$ . Elemental sulfur precipitated on the mineral surface, indicating that the dissolution of carrollite occurred via the polysulfide pathway. The surface of carrollite was selectively corroded by bacteria, and oxidation pits with different sizes were observed at various sites. Elemental sulfur, sulfate and sulfite were present on the surface of carrollite during the leaching process, and may have formed a passivation layer on mineral surface.

Key words: carrollite; bioleaching; microorganism; reaction pathway

# **1** Introduction

The Luanshya Mine is one of the most important Cu-Co deposits in Zambia and is located along the Zaire-Zambia Copperbelt, which is part of the Central African Copperbelt [1,2]. The most common cobaltbearing mineral is carrollite. The traditional technologies for extracting cobalt from the ore are pyrometallurgy and hydrometallurgy. In recent years, with the development of biohydrometallurgy, the technology of cobalt extraction by bacteria has been received much attention. Previous work showed that cobalt extraction from carrollite by bacteria was feasible [3]. Moreover, when the microbial catalyst is employed during the bioleaching process, the leaching efficiency of cobalt can increase to above 90% and the leaching time can be reduced by approximately one-third of that without the catalyst [4,5]. However, the function of the microorganism, intermediates and the reaction pathway for carrollite dissolution are not clear. Therefore, the leaching mechanism of carrollite in the presence of bacteria is desirable.

It is generally accepted that microbe mediates

bioleaching of sulfide minerals through the contact and indirect mechanisms [6,7]. The indirect mechanism occurs by  $Fe^{3+}$  ions in the solution, causing mineral oxidation. The role of the bacteria is recycling of the iron ions ( $Fe^{2+}$  to  $Fe^{3+}$ ). The contact mechanism, in contrast, requires the attachment of bacteria on the mineral surface. The attachment is predominantly mediated by the extracellular polymeric substances (EPS) surrounding the cells [6,8–10]. It is believed that the contact mechanism proceeds through a biooxidation of  $Fe^{3+}$  ions in the EPS layer [6,7,11].

Based on key intermediates and products, two separate reaction pathways for the dissolution of metal sulfides are proposed: the thiosulfate and the polysulfide pathway [6,7,12]. Metal sulfides like molybdenite, tungstenite and pyrite are considered to follow the thiosulfate pathway, as these sulfides can be exclusively oxidized via an oxidative attack by  $Fe^{3+}$  ions in the solution. Thiosulfate and polythionate are key intermediates in this leaching pathway. If the dissolution of sulfide minerals is achieved through the combined action of electron extraction by  $Fe^{3+}$  ions and a proton attack, metal sulfides such as galena, sphalerite, arsenopyrite, hauerite and chalcopyrite are considered to

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be dissolved according to the polysulfide mechanism [6]. The key intermediate is  $S^0$  in this pathway [7].

Nevertheless, despite the fact that significant progress on the metal sulfides has been achieved, the function of microorganism and reaction pathway for carrollite dissolution during bioleaching have hardly been investigated. In the present study, the function of microorganism and reaction pathways of carrollite were investigated by scanning electron microscopy (SEM), diffractometry energy X-ray (XRD), dispersive X-ray spectroscopy (EDS) and photoelectron spectroscopy (XPS).

# 2 Experimental

#### 2.1 Materials

The pure natural carrollite crystal sample was provided by the Luanshya Mine in Zambia. The chemical and XRD (D/MAX-RB, Rigaku Company, Japan) analysis results of the mineral specimens are shown in Table 1 and Fig. 1, respectively. The chemical formula of carrollite is CuCo<sub>2</sub>S<sub>4</sub>. The crystal structure of the mineral belongs to the isometric system. The crystal forms of carrollite are mainly cubic, rhombic dodecahedron, octahedron and trapezohedron. Carrollite is a hypidiomorphic crystal. The formal oxidation states of the constituent elements in CuCo<sub>2</sub>S<sub>4</sub> have been controversial for many years, with no clear consensus  $Cu^{+1}Co^{+3}-Co^{+4}S_4^{-2}$ ,  $Cu^{+1}Co_2^{+3}-S_3^{-2}S^{-1}$ , between  $Cu^{+2}Co_2^{+3}S^{-2}$  or  $Cu^{+1}Co_2^{+3}S_4^{-7}$  [13,14].

The bacteria culture used in this work designated ZY101, is a mixture of *Acidithiobacillus ferrooxidans*,

Table 1 Chemical composition of mineral specimen

Courses		Mass fra	action/%	
Source	Cu	Со	S	Others
Experimental	18.42	38.15	40.20	3.23
Theoretical	20.53	38.06	41.41	



Fig. 1 XRD pattern of mineral specimen

Acidithiobacillus thiooxidans and Leptospirillum ferrooxidans. It was cultured in 9K medium containing 3.0 g/L (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub>, 0.1 g/L K<sub>2</sub>HPO<sub>4</sub>, 0.5 g/L KCl, 0.5 g/L MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.01 g/L Ca(NO<sub>3</sub>)<sub>2</sub> and 44.3 g/L FeSO<sub>4</sub>·7H<sub>2</sub>O.

#### 2.2 Leaching experiment

300 mL ZY101 culture and 2700 mL 9K medium were mixed in a 4 L stainless steel (316L) leaching tank, and then incubated at 45 °C. After being cultured, the redox potential of the solution was about 650 mV (measured by a calomel electrode (Hg/HgCl<sub>2</sub>)). Subsequently, two carrollite samples with dimensions of 10 mm × 10 mm × 3 mm were added into the solution. One of the samples was in a special plexiglass container without lid. The container was covered by a sheet of membrane filter (pore size 1.0  $\mu$ m), which could inhibit the contact of the bacteria and mineral. The initial pH of the solution was 1.5. Water loss by evaporation was compensated with the addition of sterilized distilled water.

# 2.3 Micromorphology and composition of carrollite surface

The surface micromorphology of the carrollite sample was examined by SEM (SSX-550, Shimadzu Corporation, Japan). The chemical composition of particles formed on the mineral surface was measured by EDS (SSX-550, Shimadzu Corporation, Japan). The XPS spectrum of the carrollite surface was recorded by a surface analysis system (ESCALAB250, Thermo VG, USA). XPS measurement was performed using monochromatized Al  $K_{\alpha}$  X-ray (300 W). The pass energy is 50 eV and the energy step size is 0.1 eV. The analysis spot size is 500  $\mu$ m. The sputtering area is 2 mm  $\times$  2 mm. The core electron binding energies are given relatively to an adventitious hydrocarbon C 1s binding energy of 284.6 eV. The oxidation state of the sulfur is determined by the NIST XPS database (NIST standard database 20, Version 4.1)

#### **3 Results and discussion**

#### 3.1 Function of microorganism

Figure 2 shows the surface micromorphologies of carrollite samples after leaching for 96 h. Figure 2(a) indicates that the surface of the original sample is metallic luster and clean. In the case of non-contact of bacteria and mineral, the sample shows signs of corrosion after leaching for 96 h, as shown in Fig. 2(b). But only a few tiny etch pits and particles are observed on the specimen surface. The finding indicates that the oxidation of carrollite can occur via an oxidative attack by  $Fe^{3+}$  ions. In the case of contact of bacteria and

mineral, in contrast, the surface of specimen is seriously corroded. Some etching pits with different sizes and particles are observed on the surface, as shown in Fig. 2(c). By comparing Fig. 2(b) with (c), it is obvious that the dissolution of carrollite is significantly accelerated in the case of contact between bacteria and mineral. During leaching process, an ion-exchange between  $Fe^{3+}$  ions in solution against protons of glucuronic acid within EPS occurs, then a stable  $Fe(Glu)^{2+}$  complex is produced, as shown in Eq. (1):

$$2GluH + Fe^{3+} = Fe(Glu)_2^+ + 2H^+$$
(1)



**Fig. 2** Surface micromorphologies of carrollite samples after leaching for 96 h: (a) Original sample; (b) Non-contact; (c) Contact

As a result,  $Fe^{3+}$  ions in the solution are concentrated at the mineral/bacterial cell interface in the EPS. It is reported that the concentration of  $Fe^{3+}$  ions in EPS is approximately 53 g/L [15]. Since  $Fe^{3+}$  ions are located in the immediate vicinity of the mineral surface, this phenomenon makes the oxidation of mineral by  $Fe^{3+}$ ions in the EPS layer much quicker than by  $Fe^{3+}$  ions in the solution. It can be concluded that both indirect and contact mechanisms influence the leaching process. As bacteria and mineral contact, the dissolution of carrollite is significantly promoted. The equations for the reactions occurring during bioleaching of carrollite are summarized as follows:

$$CuCo_2S_4 + 6Fe^{3+} = Cu^{2+} + 2Co^{2+} + 6Fe^{2+} + 4S^0$$
 (2)

$$CuCo_{2}S_{4} + 6Fe^{3+\underline{Bacteria}}Cu^{2+} + 2Co^{2+} + 6Fe^{2+} + 4S^{0}$$
(3)

$$2S^{0} + 3O_{2} + 2H_{2}O^{\underline{\text{Bacteria}}} 2SO_{4}^{2-} + 4H^{+}$$
(4)

$$4Fe^{2+} + O_2 + 4H^{+\underline{\text{Bacteria}}} 4Fe^{3+} + 2H_2O$$
(5)

### 3.2 Reaction pathway

As reported, the dissolution of sulfide minerals proceeds through two separate reaction pathways: the thiosulfate pathway and the polysulfide pathway [6,7,12]. Thiosulfate and polythionates are key intermediates in the thiosulfate mechanism, and the oxidation state of the sulfur moiety in sulfide minerals is as follows:  $S^{-2} \rightarrow S^{+2} \rightarrow S^{+6}$ . In the polysulfide pathway, elemental  $S^0$  is a key intermediate, and the oxidation state of the sulfur moiety in sulfide minerals is as follows:  $S^{-2} \rightarrow S^{-6} \rightarrow S^{+6}$ .

In order to follow the oxidation of the sulfur moiety of carrollite during bioleaching, XPS analyses for elemental sulfur were conducted. The S 2p spectra from the carrollite surface after leaching with bacteria are shown in Fig. 3, and the peak parameters fitted to the spectra are listed in Table 2. Three peaks at 163.032, 162.553 and 161.477 eV fit to the spectrum of the original sample, as shown in Fig. 3(a), which are all assigned to  $S^{-2}$ . This finding indicates that the oxidation state of the sulfur moiety of carrollite is  $S^{-2}$ , which coincides with Refs. [13,14]. After leaching for 24 h, four peaks at 161.849, 162.599, 166.860 and 168.569 eV fit to the spectrum of the specimen, as shown in Fig. 3(b). The first two peaks are assigned to  $S^{-2}$ , whereas the latter two peaks are assigned to  $S^{+4}$  and  $S^{+6}$ , respectively. This indicates that sulfite and sulfate (such as jarosite) are present on the sample surface. After leaching for 72 h, four peaks at 161.641, 166.865, 168.337 and 169.535 eV fit to the spectrum of the sample (Fig. 3(d)). These peaks are assigned to  $S^0$  (161.641 eV),  $S^{+4}$  (166.865 eV), and S<sup>+6</sup> (168.337 and 169.535 eV). This indicates that element sulfur is also present on the mineral surface, besides sulfite and sulfate during the leaching process.

According to the XPS analyses, it can be concluded that the oxidation state of the sulfur moiety of carrollite is transformed as follows:  $S^{-2} \rightarrow S^{0} \rightarrow S^{+4} \rightarrow S^{+6}$ , because elemental sulfur, sulfites and sulfates are defecated. Therefore, it can be concluded that the dissolution of carrollite proceeds through polysulfide pathway, as shown in Fig. 4. The mechanism can be further explained by Eqs. (6)–(10).

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Fig. 3 XPS spectra for S 2p of carrollite surface after bioleaching for different time: (a) 0 h; (b) 24 h; (c) 48 h; (d) 72 h; (e) 96 h

$$n\text{CuCo}_{2}\text{S}_{4} + (6n-8)\text{Fe}^{3+} + 8\text{H}^{+} =$$
  
$$n\text{Cu}^{2+} + 2n\text{Co}^{2+} + 4\text{H}_{2}\text{S}_{n} + (6n-8)\text{Fe}^{2+}, \ n \ge 2 \quad (6)$$

$$H_2S_n + 2Fe^{3+} = \frac{n}{8}S_8^0 + 2Fe^{2+} + 2H^+$$
 (7)

$$\frac{1}{8}S_8^0 + 1.5O_2 + H_2O = SO_4^{2-} + 2H^+$$
(8)

$$\frac{1}{8}S_8^0 + O_2 + H_2O = SO_3^{2-} + 2H^+$$
(9)

$$2SO_3^{2-} + O_2 = 2SO_4^{2-} \tag{10}$$

#### 3.3 Dissolution of carrollite

The changes in the surface micromorphology of carrollite samples during bioleaching are shown in Fig. 4. Figure 4(a) indicated that the surface of the original sample was metallic luster and clean. After leaching for 24 h, the carrollite was corroded, and a few particles were adhered to the surface (Fig. 4(b)). After 48 h, the corrosion became more severe, and the amount of particles increased. Some etching pits with different sizes were observed on the surface (Fig. 4(c)). With increasing leaching time the corrosion faces continued to increase,

Fig. 3	Time/h	Peak	Energy/eV	Valence state	Relative content/%
(a)		1	163.032	-2	46.48
	0	2	162.553	-2	27.11
		3	161.477	-2	26.41
(b) 2		1	168.569	+6	43.56
	24	2	166.860	+4	12.86
	24	3	162.599	-2	30.36
		4	161.849	-2	13.22
(c) 48		1	168.581	+6	62.60
	19	2	166.909	+4	12.29
	40	3	162.590	-2	13.83
		4	161.485	-2	11.28
(d)	72	1	169.535	+6	16.26
		2	168.337	+6	31.74
	12	3	166.865	+4	25.84
		4	161.641	0	26.16
(e)	96	1	168.303	+6	64.48
		2	166.869	+4	8.11
		3	162.783	0	8.59
		4	161.606	0	18.82

Table 2 Peak parameters fitted to S 2p spectra

and more pits and particles on the surface became visible (Figs. 4 (d) and (e)). The size of etch pit and microcracks gradually increased. The maximum size was 0.04 mm  $\times$  0.02 mm. After 144 h, the particles increased, and a precipitate layer formed on the surface of the sample (Fig. 4(f)).

SEM images demonstrated that the carrollite surface is selectively corroded in the presence of bacteria. Some etched pits with different sizes are observed at various sites. Moreover, some etched pits are seemingly a ranged line, as shown in Figs. 4(d) and (e). There are indications from Refs. [6,16] that the attachment of bacteria on the surface of metal sulfides does not occur randomly. Bacteria preferentially attach to sites with visible surface imperfections such as scratches and low crystallization. The attachment to areas with low crystallization is favored and the sessile bacteria seem to orient along crystallographic axes.

From the data of XPS, SEM and EDS analyses, elemental sulfur, sulfate and sulfite were detected on the surface of carrollite during bioleaching. If more of these compounds adhere to the mineral surface, they can form a passivation layer on the surface of carrollite, as shown in Fig. 5. The passivation film may hinder the dissolution



**Fig. 4** Surface micromorphologies of carrollite samples after bioleaching for different time: (a) 0 h; (b) 24 h; (c) 48 h; (d) 72 h; (e) 96 h; (f) 144 h



Fig. 5 SEM image of carrollite surface after bioleaching

by inhibiting the access of oxidants (Fe<sup>3+</sup> ions) to the mineral surface [17–19].

### **4** Conclusions

1) According to the results of leaching experiment, it can be concluded that the dissolution of carrollite occurs via an oxidative attack by  $Fe^{3+}$  ions. In the presence of bacteria, since  $Fe^{3+}$  ions in the solution are concentrated at the mineral/bacterial cell interface in the EPS, the dissolution of carrollite is significantly accelerated.

2) The oxidation state of the sulfur moiety in carrollite is transformed as follows:  $S^{-2} \rightarrow S^{0} \rightarrow S^{+4} \rightarrow S^{+6}$ , and elemental sulfur, sulfate and sulfite are produced on the surface of mineral in the bioleaching process, indicating the dissolution of carrollite through the polysulfide pathway.

3) From the SEM images of carrollite samples, it is obvious that the carrollite surface is seriously corroded in the presence of bacteria, and some etch pits with different sizes are observed at various sites. Elemental sulfur, sulfates and sulfites form precipitates on the surface of carrollite. These compounds can form a passivation layer on the surface of carrollite.

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# 硫铜钴矿生物浸出过程中细菌的 作用及其溶解反应途径

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摘 要:研究硫铜钴矿生物浸出过程中细菌的作用及其溶解反应途径。结果表明,间接作用机制和接触作用机制 均对硫铜钴矿生物浸出过程产生影响。当细菌吸附到矿物表面时,矿物溶解速率显著加快,说明浸出过程中接触 作用机制对硫铜钴矿的溶解有重要影响。浸出过程中硫元素氧化价态的变化顺序为 S<sup>-2</sup>→S<sup>0</sup>→S<sup>+4</sup>→S<sup>+6</sup>,并有单质 硫沉淀在矿物表面,说明硫铜钴矿生物浸出过程按照多硫化物途径进行。硫铜钴矿表面被细菌严重腐蚀,出现许 多大小不一的腐蚀坑洞,并有单质硫、硫酸盐及亚硫酸盐生成。这些氧化产物在矿物表面形成一层钝化层。 关键词:硫铜钴矿;生物浸出;细菌;反应途径

#### (Edited by Wei-ping CHEN)