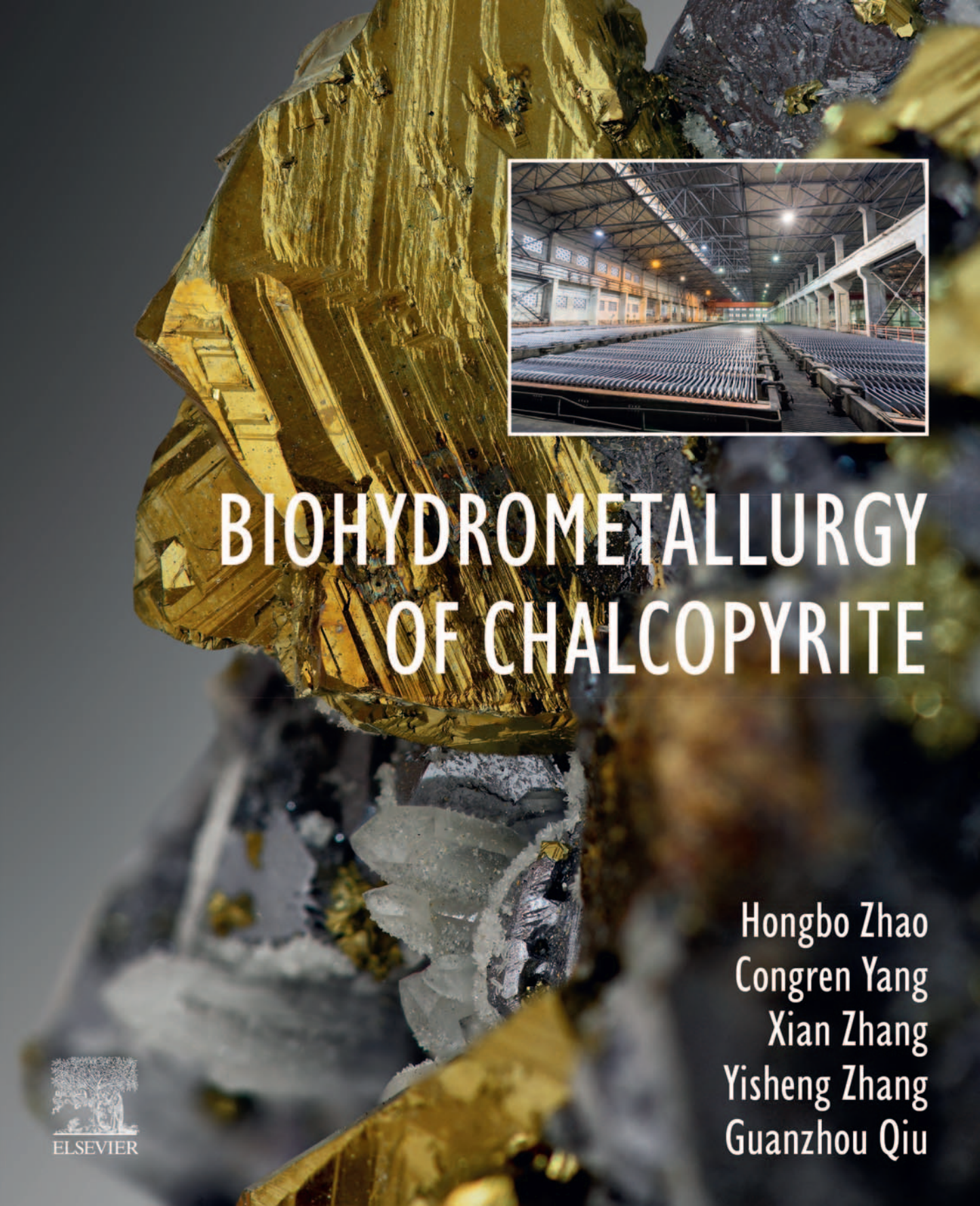




BIOHYDROMETALLURGY OF CHALCOPYRITE

Hongbo Zhao
Congren Yang
Xian Zhang
Yisheng Zhang
Guangzhou Qiu

BIOHYDROMETALLURGY OF CHALCOPYRITE

This page intentionally left blank

BIOHYDROMETALLURGY OF CHALCOPYRITE

HONGBO ZHAO

Central South University, Changsha, Hunan, China

CONGREN YANG

Central South University, Changsha, Hunan, China

XIAN ZHANG

Central South University, Changsha, Hunan, China

YISHENG ZHANG

Central South University, Changsha, Hunan, China

GUANZHOU QIU

Central South University, Changsha, Hunan, China



ELSEVIER

Elsevier

Radarweg 29, PO Box 211, 1000 AE Amsterdam, Netherlands
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States

Copyright © 2021 Elsevier Inc. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: www.elsevier.com/permissions.

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

Notices

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Library of Congress Cataloging-in-Publication Data

A catalog record for this book is available from the Library of Congress

British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library

ISBN: 978-0-12-821880-8

For information on all Elsevier publications visit our website at
<https://www.elsevier.com/books-and-journals>

Publisher: Candice Janco

Acquisitions Editor: Kostas KI Marinakis

Editorial Project Manager: Ruby Smith

Production Project Manager: Kumar Anbazhagan

Cover Designer: Miles Hitchen

Typeset by TNQ Technologies



Contents

Preface vii

1. Microorganisms used in chalcopyrite bioleaching

- 1.1 Overview of bioleaching microorganisms 2
- 1.2 Structure composition and functional diversity of microbial communities 3
- 1.3 Functional role of coexisting bacteria in bioleaching systems 14
- 1.4 Ecology and metabolism of sulfur- and/or iron-oxidizing *Acidithiobacillus* 30
- Acknowledgments 41
- References 41

2. Properties of chalcopyrite

- 2.1 Oxidation state of Cu, Fe, and S elements 51
- 2.2 Electronic structure 57
- 2.3 Reconstruction of chalcopyrite surfaces 60
- 2.4 Summary 69
- Acknowledgments 70
- References 70

3. Electrochemical dissolution process of chalcopyrite

- 3.1 Electrochemical behavior of chalcopyrite 73
- 3.2 Surface species of chalcopyrite after treatment by different potentials 87
- 3.3 Disulfide and polysulfide passivation of the chalcopyrite surface 94
- 3.4 Optimum range of redox potential for chalcopyrite leaching 98
- 3.5 Enhancing bioleaching of chalcopyrite by controlling the solution potential 112
- 3.6 Summary 118

- Acknowledgments 120
- References 120

4. Dissolution and passivation mechanism of chalcopyrite in bioleaching

- 4.1 Passivation mechanism of chalcopyrite bioleaching 125
- 4.2 Effects of mineralogical properties on passivation and dissolution mechanism 135
- 4.3 Brief overview of effects of mineralogical properties 145
- 4.4 Summary 149
- Acknowledgments 150
- References 151

5. Role of gangue minerals in chalcopyrite bioleaching

- 5.1 Role of marmatite in chalcopyrite bioleaching 157
- 5.2 Role of pyrite in chalcopyrite bioleaching 172
- 5.3 Other gangue minerals 177
- 5.4 Summary 178
- Acknowledgments 179
- References 179

6. Chalcopyrite bioleaching catalyzed by silver

- 6.1 Catalytic mechanism of silver ions in chalcopyrite bioleaching 183
- 6.2 Catalyzed bioleaching using silver-containing waste 201
- 6.3 Summary 205
- Acknowledgments 206
- References 206

7. Industrial application for chalcopyrite bioleaching	7.4 Summary 226 Acknowledgments 226 References 226
7.1 Bioleaching technology 211	
7.2 Industrial practice cases 214	Index 233
7.3 Key parameters and controlling techniques 214	

Preface

Chalcopyrite (CuFeS_2) is the most abundant copper-containing mineral resource in the world, accounting for more than 70% of global copper reserves. It is also widely distributed in solid waste and secondary resources. Chalcopyrite is mainly treated by beneficiation-pyrometallurgy technology to recover copper, which cannot process chalcopyrite of a low grade and fine and complex distribution easily and economically. Biohydrometallurgy (bioleaching, biomining, microbial leaching, and bacterial leaching) is an alternative hydrometallurgical technology for conventional beneficiation-pyrometallurgy, especially used to process mineral resources of low grade and fine and complex distribution. It also has an important role in processing solid waste and secondary resources, such as tailings, smelter waste, and electronic waste.

It is important to develop bioleaching technology for chalcopyrite, especially heap leaching. However, the large-scale industrialization of chalcopyrite bioleaching is hindered by its slow leaching kinetics and low

copper extraction. To overcome this challenge, the dissolution mechanism of chalcopyrite bioleaching should first be interpreted, and then control and intensification techniques need to be developed. In this book, the related information is summarized and discussed in seven chapters, including microorganisms used in chalcopyrite bioleaching ([Chapter 1](#)), the properties of chalcopyrite ([Chapter 2](#)), the electrochemical dissolution process of chalcopyrite ([Chapter 3](#)), the dissolution and passivation mechanism of chalcopyrite in bioleaching ([Chapter 4](#)), the role of gangue minerals in chalcopyrite bioleaching ([Chapter 5](#)), the catalyzed bioleaching of chalcopyrite by silver ([Chapter 6](#)), and industrial applications for chalcopyrite bioleaching ([Chapter 7](#)). We believe that this book will be a useful reference in both laboratory research and industrial production.

All the authors
Central South University,
Changsha, Hunan, China

This page intentionally left blank

Microorganisms used in chalcopyrite bioleaching

The spatiotemporal distribution of populations in various eco-niches is thought to be potentially related to individual differences in the use of nutrients or other resources, but their functional roles in the microbial communities remain elusive. We compared differentiation in gene repertoire and metabolic profiles, with a focus on the potential functional traits of three commonly recognized members (*Acidithiobacillus caldus*, *Leptospirillum ferriphilum*, and *Sulfobacillus thermosulfidooxidans*) in bioleaching heaps. Comparative genomics revealed that these cooccurring bacteria shared a few homologous genes, which significantly suggested genomic differences among these organisms. Notably, relatively more genes assigned to the Clusters of Orthologous Groups category [G] (carbohydrate transport and metabolism) were identified in *S. thermosulfidooxidans* compared with the two other species, which probably indicated their mixotrophic capabilities that assimilate both organic and inorganic forms of carbon. Further inspection revealed distinctive metabolic capabilities involving carbon assimilation, nitrogen uptake, and iron-sulfur cycling, providing robust evidence for functional differences with respect to nutrient use. Therefore, we proposed that the mutual compensation of functionalities among these cooccurring organisms might provide a selective advantage for efficiently using the limited resources in their habitats. Furthermore, it might be favorable to chemoautotrophs' lifestyles to form mutualistic interactions with these heterotrophic and/or mixotrophic acidophiles, in which the latter could degrade organic compounds to detoxify the environments effectively. Collectively, the findings shed light on the genetic traits and potential metabolic activities of these organisms and enable us to make some inferences about genomic and functional differences that might allow them to coexist. Many scientific studies revealed that the acidophilic ferrous and/or sulfur oxidizers, *Acidithiobacillus* spp., were widely found in diverse mine tailings with low pH and a high concentration of toxic substances; thus, it is necessary to identify the cellular mechanisms to cope with these harsh environmental conditions. Pan-genome analysis of 10 bacteria belonging to the genus *Acidithiobacillus* indicated that all strains shared a large number of core genome, most of which were assigned to the metabolism-associated genes. In addition, unique genes of *Acidithiobacillus ferrooxidans* were much lesser than those of other species. In particular, *Acidithiobacillus ferrivorans*—specific genes with a large

proportion were mapped to metabolism-related genes, indicating that diverse metabolic pathways might confer an advantage to adapt to local environmental conditions. Analyses of functional metabolisms revealed the differences of carbon metabolism, nitrogen metabolism, and sulfur metabolism at the species and/or strain level. Comparison across species and/or strains of *Acidithiobacillus* populations brings a deeper appreciation of metabolic differences and highlights the importance of cellular mechanisms that maintain the basal physiological functions under complex environments.

1.1 Overview of bioleaching microorganisms

In industry, biohydrometallurgy has been successfully exploited to extract basic metals from sulfide minerals (Demergasso et al., 2005). In the field of industrial applications, mine tailings of a low grade were commonly used for bioleaching heaps. During bioleaching operations, insoluble metal sulfides were converted into water-soluble metal sulfates, resulting in considerably high concentrations of soluble iron and a low pH. Ferrous iron (Fe[II]) was oxidized rapidly to generate ferric iron (Fe[III]) under oxygen-saturated conditions with neutral pH, whereas in acidic environments, Fe(II) was stable even under the condition of atmospheric oxygen (O₂). Thus, Fe(II) could be used by microbial communities as the electron donor (Bonnefoy & Holmes, 2012). Nonetheless, the highest levels of soluble iron could threaten microorganisms inhabiting these environments. Hydroxyl radicals produced by the reaction of free ferrous iron with oxygen (Fenton reaction) would damage biological macromolecules and even cause cell death (Touati, 2000). Thus, specialized environments provide a particular opportunity and a potential challenge for acidophilic life.

These habitats with a low pH and high concentration of toxic substances represent a type of extremely acidic environment of anthropogenic origin (Bonnefoy & Holmes, 2012) that are inhospitable to most prokaryotes and eukaryotes (Guo et al., 2014; Yelton et al., 2013). However, in both natural and man-made extremely acidic environments (pH < 3), there is a considerable diversity of acidophilic microorganisms (mostly Eubacteria and Archaea) (Bonnefoy & Holmes, 2012; Johnson, 1998). Acidophiles that have a pH optimum less than 3 are endowed with the peculiar adaptive mechanisms and survivability to thrive in these extreme environments. Acidophilic prokaryotes typically associated with bioleaching operations were widely studied, primarily because of their potential for commercial application. Much original research and many review articles have focused on microbial species and their biological nature. Generally, acidophilic prokaryotic microorganisms are classified as iron oxidizers, sulfur oxidizers, iron-and-sulfur oxidizers, iron reducers, iron oxidizers/reducers, iron oxidizers/reducers and sulfur oxidizers, heterotrophic acidophiles, and obligate anaerobes (Johnson & Hallberg, 2003). These bacteria live over a wide temperature range and include psychrotolerant but not psychrophilic species that survive at temperatures as low as 4°C, mesophiles (between 20 and 40°C), moderate thermophiles (between 40 and 60°C), and extreme thermophiles (above 60°C) (Bonnefoy & Holmes, 2012; Johnson, 1998; Johnson & Hallberg, 2003).

Unraveling the ecological and functional roles of microorganisms in biological communities is an important but still elusive issue (Prosser et al., 2007), although these microorganisms are thought to be crucial to the function of ecosystems (Harris, 2009; Hua et al., 2015; Jiao et al.,

2010). As stated by Sogin et al. (Sogin et al., 2006), there is a surprisingly wide biodiversity of microbial communities in pristine environments. In their study, the dominant populations are numerically significant, but members of the rare biosphere account for most of the phylogenetic diversity. Similar results were generally observed in other natural and anthropogenic environments based on metagenomic and metatranscriptomic analyses (Chen et al., 2015; Goltsman, Comolli, Thomas, & Banfield, 2015; Xiao et al., 2016; Zhang et al., 2016d). Genomes of microbial members in various communities have been reconstructed with the benefit of cultivation-independent sequencing (Mason et al., 2012; Tyson et al., 2004; Wu et al., 2016), providing a first glimpse of their functional roles in situ. In addition, several bioinformatics-based strategies have been attempted to obtain genomic assemblies from metagenomic datasets (Dick et al., 2009; Hua et al., 2015).

1.2 Structure composition and functional diversity of microbial communities

In recent decades, issues associated with microbial life in oligotrophic, extremely acidic environments have been discussed in a large number of reviews and papers, including the occurrence and composition of microbial communities (González-Toril, Llobet-Brossa, Casamayor, Amann, & Amils, 2003; López-Archilla, Marin, & Amils, 2001; Sánchez-Andrea, Rodríguez, Amils, & Sanz, 2011), their strategies to tolerate metal and a low pH (Baker-Austin & Dopson, 2007; Franke & Rensing, 2007), as well as their metabolisms and functions (Sabater et al., 2003; Tyson et al., 2004). In particular, microbial communities in mine tailings have attracted considerable interest, and there is much relevant microbiological research related to mine tailings (Chen et al., 2013; Schippers et al., 2010). Previous cultivation-dependent studies of mine tailings in several countries have revealed a numerical dominance of Bacteria over Archaea. Abundant microorganisms are acidophilic iron- and/or sulfur-oxidizing *Acidithiobacillus* and *Leptospirillum* (Bosecker, Mengel-Jung, & Schippers, 2004; Breuker, Blazejak, Bosecker, & Schippers, 2009; Kock & Schippers, 2006, 2008). In addition, metagenomic analysis revealed that the most abundant microorganisms in a low-temperature acid mine drainage (AMD) stream were most similar to the psychrotolerant acidophile, *A. ferrivorans* (Liljeqvist et al., 2015). However, studies on extremely acidic lead/zinc mine tailings revealed that acidophilic Archaea, mostly ferrous-iron-oxidizing *Ferroplasma acidophilum*, were numerically significant, indicating their importance in extremely acidic environments (Huang et al., 2011).

The microbial ecology of full-scale heap or dump bioleaching of copper ore has been poorly understood (Brierley, 2001), whereas an understanding of microbiological components of bioheaps facilitated commercial bioheap applications. Although 16S ribosomal RNA (rRNA) gene analysis was targeted as a useful method in many studies of extreme environments, the development of sequencing technology, metagenomics methods, and bioinformatics tools has provided a valuable platform for environmental gene pool identification and potential functional prediction of biogeochemical relevance in microbial populations (Johnson, Chevrete, Ehlmann, & Benison, 2015). Metagenomics, or the culture-independent genomic analytical method of microorganisms, was a powerful approach to capture the entire spectrum of microbial communities including both cultivatable and uncultivable microorganisms, the latter of which could not be cultured by standard techniques but comprised the majority of

biological diversity (Friedrich, 2005; Handelsman, 2004; Riesenfeld, Schloss, & Handelsman, 2004; Streit & Schmitz, 2004). Metagenomic research associated with ecological roles of uncultured and rare microorganisms showed their importance in AMD communities (Hua et al., 2015). A combination of shotgun metagenome sequencing and computational approaches for genome assembly has advanced to metagenomics, providing glimpses into the uncultured microbial world (Schloss & Handelsman, 2005).

In this section, we collected samples from the surface-layer mine tailings of the bioleaching heap located in the Dexing Copper Mine, Jiangxi Province, China. By investigating the taxonomic classification and functional genes involved in several key metabolic processes, based on metagenome analyses, we sought to characterize the microbial community composition in the bioleaching dumps heaped by mine tailings and the functional coding potential of microorganisms related to key metabolic pathways within extremely acidic environmental conditions.

1.2.1 Sequencing, de novo assembly, gene prediction, and functional annotation

Metagenomic DNA was subjected to Illumina MiSeq sequencing. Approximately 3.4 million short DNA sequences were then used for bioinformatics analysis. After quality control using an NGS QC Toolkit, 2,941,297 (87.80%) reads with high quality were obtained (Table 1.1). Subsequently, all of these high-quality reads were assembled, and a self-writing script was used to filter the assembled sequences under 300 base pairs (bp), resulting in a total of 301,907,459 bp, with an N50 of 641 bp (481,688 contigs ranging from 301 to 49,868 bp; mean length, 626 bp). For gene prediction, 660,572 coding sequences (CDS) were identified using the program MetaGeneAnnotator.

All putative protein coding sequences were searched against databases including National Center for Biotechnology Information-non-redundant protein sequence database (NCBI-nr), the extended Clusters of Orthologous Groups (COG) (Franceschini et al., 2013), and Kyoto Encyclopedia of Genes and Genomes (KEGG). We obtained a total of 535,887 (81.12%), 517,948 (78.41%), and 494,721 (74.89%) significant Basic Local Alignment Search Tool (BLAST) hits, respectively. Moreover, 497,601 sequences (75.33%) and 261,595 sequences (39.60%) were assigned to the COG categories and KEGG Orthology, respectively (Table 1.1). Among the 25 COG categories, metagenome sequences were assigned to 23 of them (Fig. 1.1). A large proportion of sequences were assigned to the COG category [S] (function unknown) (80,561 CDSs; 16.19%) and COG category [R] (general function prediction only) (39,507 CDSs; 7.94%), indicating large pools of potential unknown functional genes in copper bioleaching operations. Furthermore, the large amount of genes associated with basal metabolisms such as amino acid transport and metabolism (COG category [E]) and energy metabolism (COG category [C]) indicated the ubiquitous substance and energy metabolism in the extremely environments, maintaining basic microbial activities.

1.2.2 Taxonomic assignment of metagenome datasets

To reveal metagenome sequence classification of microbial communities in tailings sample, taxonomic analyses at the genus level were performed. Taxonomic assignment using the program MEGAN revealed unexpectedly abundant microbial biodiversity (over 100 genera)

TABLE 1.1 Metagenome dataset of samples from surface-layer mine tailings.

Properties	Values
Raw sequence reads	3,349,899
High-quality sequences	2,941,297 (87.80%)
Assembly sequences	481,688
Total bases	301,907,459
Maximum sequence length (bp)	49,868
Minimum sequence length (bp)	301
Mean length (bp)	626
Mean guanine plus cytosine (%)	60.00
N50 (bp)	641
Protein-coding sequences	660,572
CDSs with NCBI-nr hits	535,887 (81.12%)
CDSs with COG hits	517,948 (78.41%)
CDSs with COGs	497,601 (75.33%)
CDSs with KEGG hits	494,721 (74.89%)
CDSs with KEGG Orthology	261,595 (39.60%)

CDSs, coding sequences; COG, Clusters of Orthologous Groups.

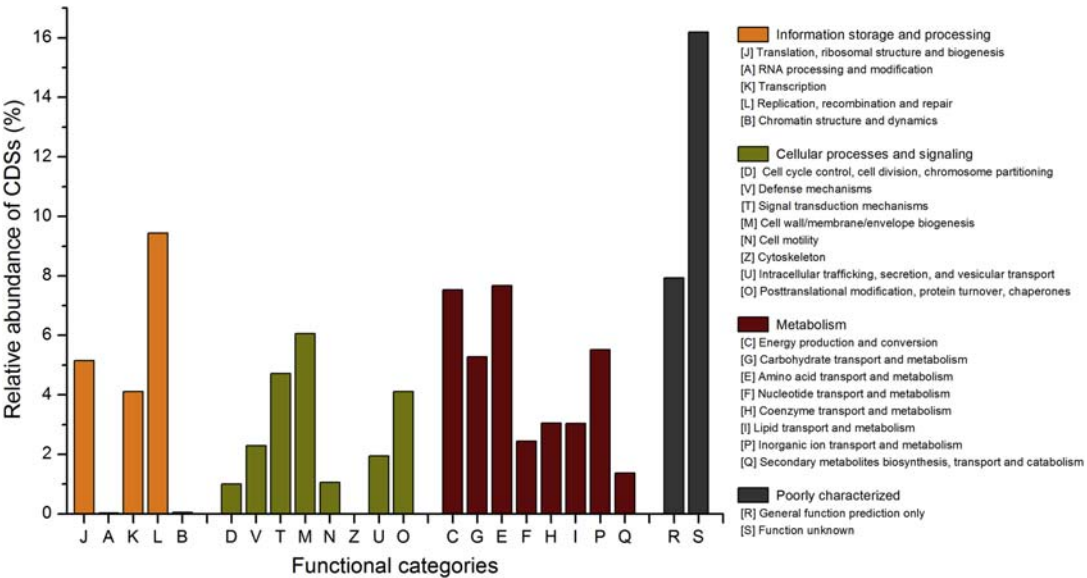


FIGURE 1.1 The clusters of orthologous groups (COG) categories of metagenome data from mine tailings. From Zhang, X., Niu, J., Liang, Y., Liu, X, Yin, H. (2016). *Metagenome-scale analysis yields insights into the structure and function of microbial communities in a copper bioleaching heap*. BMC Genetics, 17(1), 21. <https://doi.org/10.1186/s12863-016-0330-4>.

of this extreme environments (surface-layer of copper mine tailings) to some extent, which hindered sequence assembly owing to the low sequencing depth. Copper mine tailings in this study harbored diverse microbial populations, possibly because of various niches related to gradients of physicochemical conditions, which were discussed previously in AMD environments (Baker & Banfield, 2003; Chen et al., 2013; Hallberg, 2010; Johnson & Hallberg, 2003). MEGAN analysis showed that the microbial community in the mineral surface-layer was dominated by the sulfur- and iron-oxidizing acidophiles of *Acidithiobacillus*-related and *Leptospirillum*-related groups (Fig. 1.2).

In these *Acidithiobacillus*-related sequences, most were assigned to *A. ferrivorans*, followed closely by *A. ferrooxidans*. In the extremely acidic tailings, approximately 93.47% of the total *Acidithiobacillus*-related sequences were affiliated with *A. ferrivorans* and *A. ferrooxidans*. As a major participant of iron- and sulfur-oxidizing acidophilic bacteria, *A. ferrivorans* has been widely found in metal mine-affected environments (Hallberg, 2010). Likewise, *A. ferrooxidans*, which used energy from the oxidation of sulfur- and iron-containing minerals, was a principal member in consortia of microorganisms associated with the bioleaching or biomining (industrial recovery of copper) (Valdés et al., 2008a). The numerical dominance of *Acidithiobacillus*-related sequence indicated its importance in the surface layer of copper mine tailings during industrial bioleaching operations. Moreover, *Rhodanobacter* (7.34%), *Thiobacillus* (6.03%), *Leptospirillum* (5.57%), and *Acidiphilium* (4.51%) were found in surface-layer mine tailings. In addition, 82 CDSs were assigned to virus, most of which were affiliated with the double-stranded DNA viruses with no RNA stage. Of these sequences, most taxonomic hits (74%) shared sequence identity with sequences in the order *Caudovirales*, based on the taxonomy of viral genomes provided by the GenBank

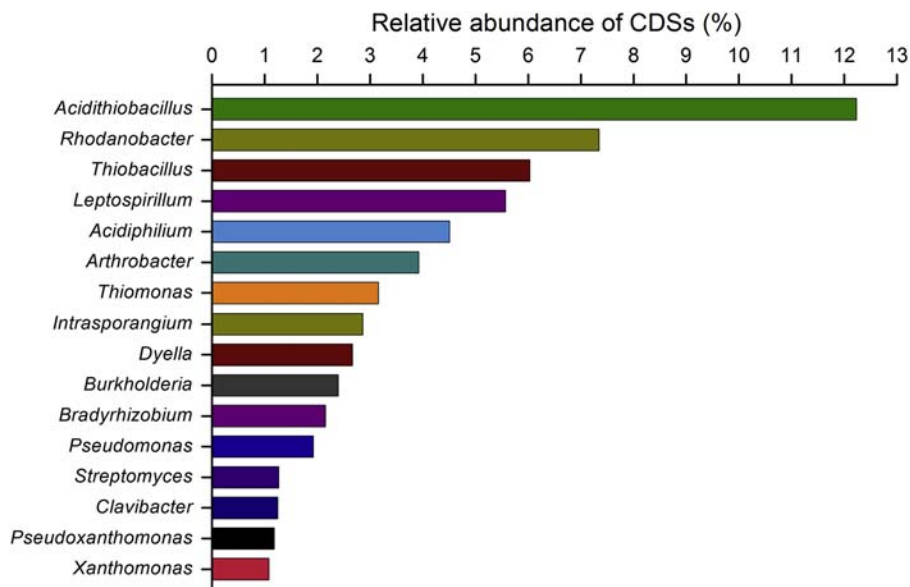


FIGURE 1.2 Taxonomic composition analysis at the genus level based on contigs sequences (≥ 300 base pairs) in the metagenome dataset. Only genera with the specified percentage abundance ($\geq 1\%$) are shown. CDSs, coding sequences. From Zhang, X., Niu, J., Liang, Y., Liu, X., Yin, H. (2016). Metagenome-scale analysis yields insights into the structure and function of microbial communities in a copper bioleaching heap. *BMC Genetics*, 17(1), 21. <https://doi.org/10.1186/s12863-016-0330-4>.

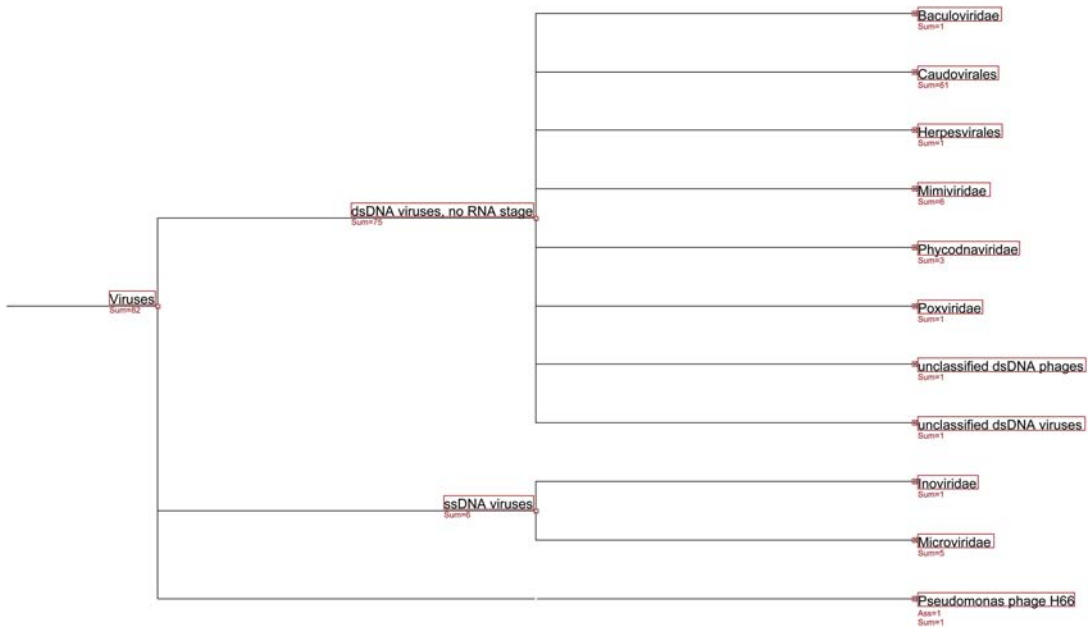


FIGURE 1.3 Viral composition in the metagenome collected from the bioleaching heap. *dsDNA*, double-stranded DNA; *ssDNA*, single-stranded DNA. From Zhang, X., Niu, J., Liang, Y., Liu, X., Yin, H. (2016). Metagenome-scale analysis yields insights into the structure and function of microbial communities in a copper bioleaching heap. *BMC Genetics*, 17(1), 21. <https://doi.org/10.1186/s12863-016-0330-4>.

database (Fig. 1.3). This was consistent with the viruses previously described from the desert (Adriaenssens et al., 2015; Fancello et al., 2013) and metaviromes from other environments such as marine (Breitbart et al., 2002).

Depending on the automated analysis pipeline implemented in the MG-RAST platform, the microbial populations at the phylum level were phylogenetically assigned to Proteobacteria, Actinobacteria, Nitrospirae, Bacteroidetes, Gemmatimonadetes, Acidobacteria, Firmicutes, Deinococcus-Thermus, Euryarchaeota, and several other phyla mainly belonging to the domain Bacteria (Fig. 1.4). In more detail, Proteobacteria-related sequences with the most abundance were composed of the class Gammaproteobacteria, Betaproteobacteria, Alphaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria, and Zetaproteobacteria, in order from highest to lowest. Similarly, community diversity analysis based on a polymerase chain reaction–based cloning approach showed that most sequenced clones were affiliated with the Gammaproteobacteria (Yin et al., 2008). Because the most abundant microbes were similar to the *Acidithiobacillus*-like genus, it was proposed to belong to the new class *Acidithiobacillia* (a sister group of class Gammaproteobacteria) (Williams & Kelly, 2013). Thus, the most abundant sequences at the class level in this extreme environment could be assigned to *Acidithiobacillia*-related microorganisms. The phylum *Euryarchaeota* occupied the largest proportion in domain Archaea. However, it was relatively low in the whole metagenome dataset, which suggests that the Archaea might have little role in the surface layer of copper mine tailings.

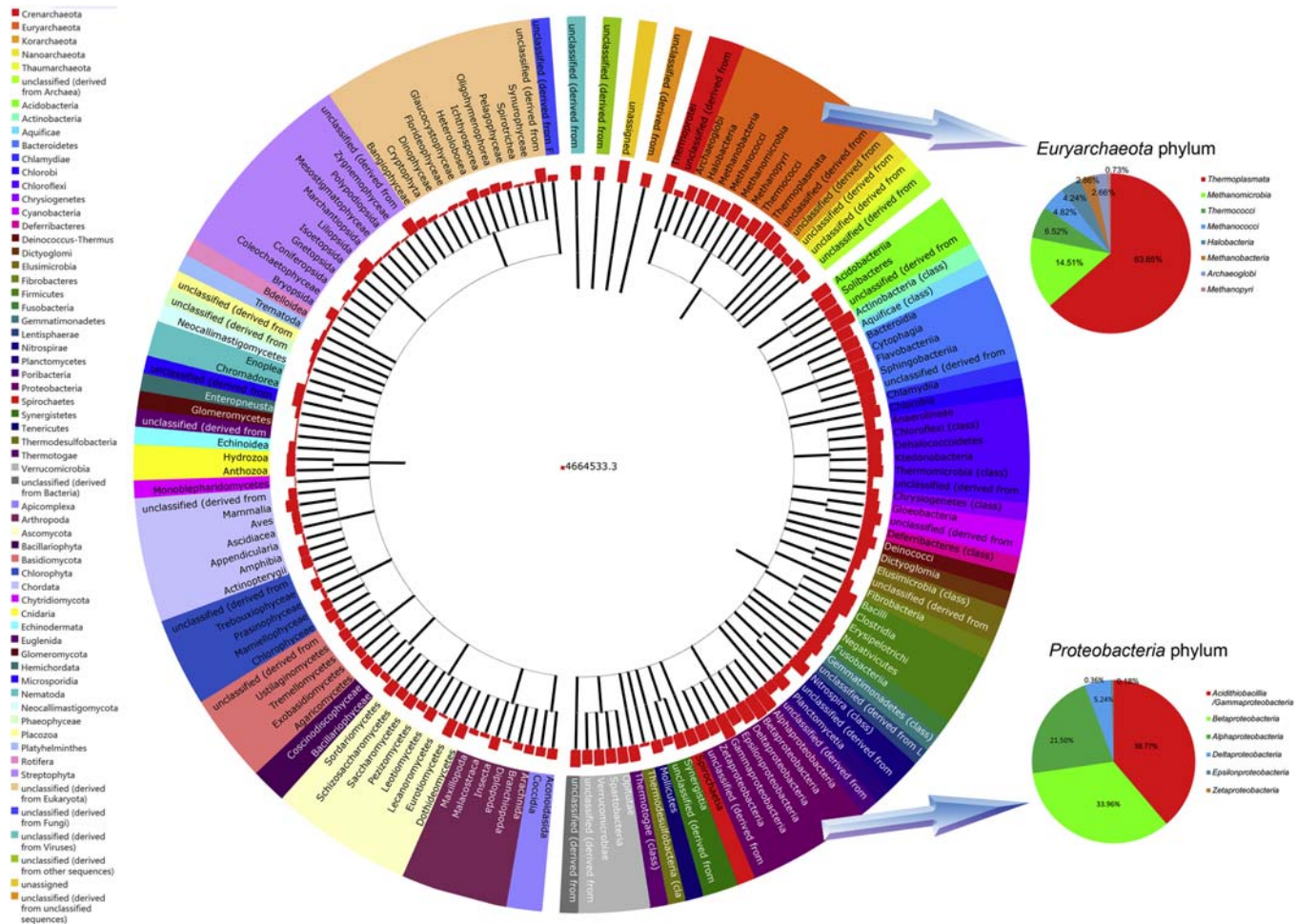


FIGURE 1.4 Phylogenetic tree at the phylum level based on the metagenome dataset. The data were compared with M5NR using a maximum E-value of $1e-5$, a minimum identity of 60%, and a minimum alignment length of 15 measured in aa for protein and bp for RNA databases. In addition, leaf abundance weights are displayed as stacked bar charts. The maximum taxonomical level is class and the leaves are colored by phylum. From Zhang, X., Niu, J., Liang, Y., Liu, X., Yin, H. (2016). Metagenome-scale analysis yields insights into the structure and function of microbial communities in a copper bioleaching heap. *BMC Genetics*, 17(1), 21. <https://doi.org/10.1186/s12863-016-0330-4>.

1.2.3 Key genes coding for enzymes associated with principal metabolisms

The vital activities of the chemolithotrophy-based microbial community present in mine tailings mainly rely on metabolic capabilities to metabolize carbon, nitrogen, iron, and sulfur. Thus, it is necessary to investigate the general metabolisms of microbial processes, aiming to understand the subcycling of those elements within a copper bioleaching heap.

1.2.3.1 Autotrophic carbon fixation

Cellular carbon acquired from inorganic carbon is essential for life, suggesting the transition of carbon from the inorganic to organic world. Research revealed that six different pathways for carbon fixation exist in microorganisms, including the Calvin–Benson–Bassham (CBB) cycle, reductive citric acid (rTCA) cycle, reductive acetyl-coenzyme A (acetyl-CoA) pathway, 3-hydroxypropionate bicycle, 3-hydroxypropionate/4-hydroxybutyrate cycle (hydroxypropionate–hydroxybutyrate cycle), and dicarboxylate–4-hydroxybutyrate cycle (dicarboxylate–hydroxybutyrate cycle) (Berg et al., 2010; Chen et al., 2013). Functional annotation against databases (i.e., NCBI-nr, the extended COG, and KEGG) showed that almost all genes encoding for CBB cycle and rTCA cycle were identified, whereas no gene involved in the other four pathways for carbon fixation was identified. This finding was largely consistent with previous studies (Chen et al., 2013).

In the CBB cycle, there is a series of enzymatic reactions, one of which is involved in CO₂ fixation catalyzed by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco); the others are responsible for regenerating ribulose 1,5-bisphosphate (Levicán, Ugalde, Ehrenfeld, Maass, & Parada, 2008). In addition, the key enzymes for the CBB cycle are Rubisco and phosphoribulokinase (or ribulose-5-phosphate kinase) (Berg et al., 2010; Shively, Van Keulen, & Meijer, 1998). Rubisco exists in various forms in diverse organisms from all domains of life (Guo et al., 2013). Our results indicated that genes coding for Rubisco were enriched in the metagenome, most of which were affiliated with *Acidithiobacillus*-like populations. Indeed, genomic as well as proteomic evidence showed that *Acidithiobacillus* occupied an important position in carbon fixation in AMD (Bertin et al., 2011). In addition, most phosphoribulokinase genes were from *Thiobacillus*-like microorganisms, indicating its importance in the bioleaching system. However, no gene encoding the sedoheptulose-1,7-bisphosphatase (SBPase), which catalyzed sedoheptulose 1,7-bisphosphate to generate sedoheptulose 7-phosphate, was found in the metagenome. The lack of such genes was likely due to the low sequencing depth. Besides, the candidate genes that probably presented in this metagenome dataset were not yet identified because of our limited knowledge of SBPase genes within the acidophiles.

Autotrophic CO₂ fixation via the rTCA cycle was considered an important pathway in microbial communities (Hügler, Wirsén, Fuchs, Taylor, & Sievert, 2005). Two key enzymes (2-oxoglutarate ferredoxin oxidoreductase and adenosine triphosphate (ADP) citrate lyase) are related to the rTCA cycle (Berg et al., 2010). In some species, the citrate cleavage pathway could be catalyzed by other two enzymes, citryl-CoA synthetase (EC 6.2.1.18) and citryl-CoA lyase (EC 4.1.3.34), instead of ATP citrate lyase (ACL; EC 2.3.3.8) (Berg, 2011). However, these two enzymes (i.e., citryl-CoA synthetase and citryl-CoA lyase) were phylogenetically related to ACL (Aoshima, Ishii, & Igarashi, 2004; Hügler, Huber, Molyneaux, Vetriani, & Sievert, 2007). Furthermore, research indicated that ACL was formed by a gene fusion of citryl-CoA lyase and citryl-CoA synthetase (Hügler et al., 2007). These findings could explain why no genes encoding ACL were identified in previous studies on lead-zinc mine tailings (Chen et al., 2015).

In *L. ferriphilum*, a complete set of enzymes involved in rTCA cycle was found (Levicán et al., 2008). Based on this research, our results in this study showed that gene homologs for many steps in the rTCA cycle were assigned to the *L. ferriphilum*-like populations. As for citryl-CoA synthetase, however, the small subunit gene (*ccsB*) was not detected.

1.2.3.2 Nitrogen metabolism

As the main nitrogen sources, generally speaking, atmospheric nitrogen, nitrate, nitrite, ammonium, and glutamine are used widely by microbes in natural environments. Six subsystems related to the nitrogen metabolisms, including nitrogen fixation, dissimilatory nitrate reduction, assimilatory nitrate reduction, denitrification, nitrification, and anammox, were discussed in previous papers (He et al., 2010; Mason et al., 2014). In this metagenome, a large number of genes involved in nitrogen fixation, including *nifD*, *nifK*, *nifH*, *nifE*, *nifN*, and *nifX*, which formed a *nif* operon encoding the Mo–Fe nitrogenase enzyme complex (Méndez-García et al., 2015), were detected. The relevant genes were largely found in *Leptospirillum*-like and had the highest similarity to those from *Leptospirillum ferrooxidans*-like species, suggesting the vital position of *Leptospirillum*-like organisms in bioleaching systems with limited fixed nitrogen from external sources.

Nitrogen in the form of ammonium could be directly assimilated into biomass or oxidized by two key enzymes involved in nitrification (ammonium monooxygenase and hydroxylamine oxidoreductase, which were encoded by the *amoCAB* operon and *hao* gene, respectively) (Méndez-García et al., 2015). In this metagenome, however, the lacking *hao* gene suggested that ammonium could not be used through nitrification. Mine tailings might contain elevated concentrations of nitrate caused by nitrogen-based explosives. As for dissimilatory nitrate reduction and assimilatory nitrate reduction, most genes encoding metabolic enzymes were related to those from *Thiobacillus*-like, *Acidithiobacillus*-like, and *Leptospirillum*-like populations (Fig. 1.5). Because there was limited fixation nitrogen from the atmosphere and a relatively low content of available nitrogen in mine tailings, it was speculated that these microorganisms probably had an important role in maintaining the nitrogen sources of microbial communities by using nitrate and nitrite.

Four enzymes are reported to be involved in denitrification (i.e., nitrate reductase, nitrite reductase [NO-forming], nitric oxide reductase [cytochrome *c*], and nitrous oxide reductase), which are encoded by *nar* operon, *nirK*, *norBC*, and *nosZ*, respectively (Chen et al., 2013; Jones, Stres, Rosenquist, & Hallin, 2008). In this process, nitrate or nitrite ions could be used as terminal electron acceptors under anoxic or low-oxygen conditions (Méndez-García et al., 2015). In addition, incomplete ammonification in this system, which lacks relevant genes encoding hydrazine oxidoreductase (Fig. 1.5), presumably indicates that organic nitrogen compounds in the environment cannot be degraded by microbes in the bioleaching system.

1.2.3.3 Ferrous iron oxidation

Under acidic conditions, ferrous iron in a stable status (no matter whether atmospheric oxygen exists) could be used by microorganisms (Bonnefoy & Holmes, 2012). Widespread distribution of ferrous iron oxidation capabilities has been observed in acidophilic Bacteria and Archaea (Emerson, Fleming, & McBeth, 2010; Hedrich, Schlömann, & Barrie Johnson, 2011; Weber, Achenbach, & Coates, 2006). The ability to oxidize iron has been widely distributed in microbes from neutral pH environments. Details of ferrous iron oxidation and electron transport pathways are available for the gram-negative bacterium *A. ferrooxidans* previously

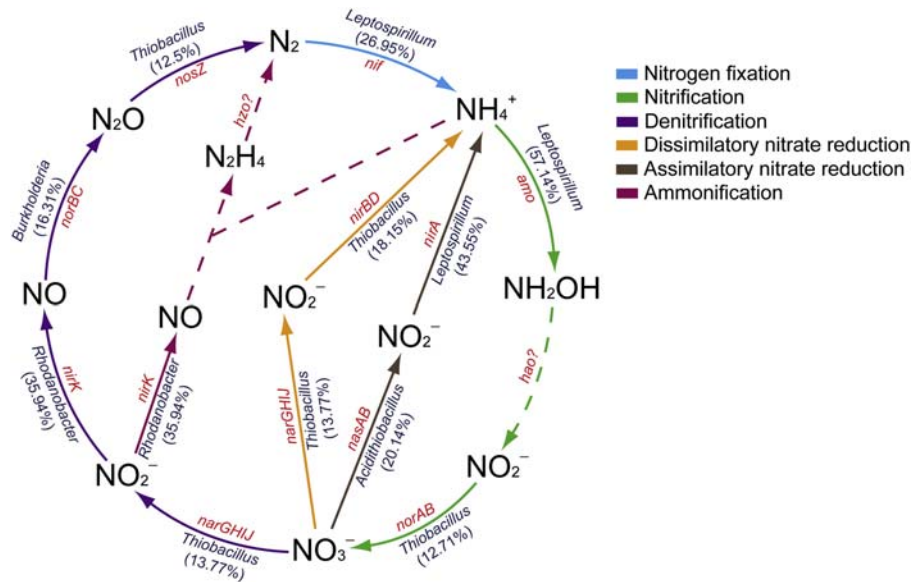


FIGURE 1.5 Schematic diagram of nitrogen metabolism in surface-layer mine tailings representing an important part of bioleaching system. Based on metagenomic data, solid lines indicate the presence of protein-coding genes associated with six major pathways, whereas dashed lines show that no gene was found in this metagenome. Different colored lines depict various metabolic pathways. Most main implicated taxa and groups governing the enzymatic reactions are displayed, and the percentages of coding sequences related to each group are shown. *amo*, ammonia monooxygenase; *hao*, hydroxylamine dehydrogenase; *hzo*, hydrazine oxidoreductase; *narGHII*, nitrate reductase (dissimilatory); *nasAB*, nitrate reductase (assimilatory); *nif*, nitrogenase (various subunits); *nirA*, ferredoxin-nitrite reductase; *nirBD*, nitrite reductase (NADH); *nirK*, nitrite reductase (NO-forming); *norAB*, nitrate reductase (A and B represent alpha subunit and beta subunit, respectively); *norBC*, nitric oxide reductase; *nosZ*, nitrous oxide reductase. From Zhang, X., Niu, J., Liang, Y., Liu, X., Yin, H. (2016). Metagenome-scale analysis yields insights into the structure and function of microbial communities in a copper bioleaching heap. *BMC Genetics*, 17(1), 21. <https://doi.org/10.1186/s12863-016-0330-4>.

identified as affiliated with the class Acidithiobacillia (Williams & Kelly, 2013). Moreover, iron oxidation pathways in other known acidophilic prokaryotes (e.g., *A. ferrovorans*, *L. ferrooxidans*, and *Thiobacillus prosperus*, which was reclassified as *Acidihalobacter prosperus*) (Cárdenas, Ortiz, Norris, Watkin, & Holmes, 2015) have been studied (Bonnefoy & Holmes, 2012). The results showed that there were no relevant genes for the *pio* operon (*pioA*, *pioB*, and *pioC*) or the *fox* operon (*foxE*, *foxY*, and *foxZ*) in the metagenome, which were reported to be involved in phototrophic iron oxidation in *Rhodopseudomonas palustris* and *Rhodobacter capsulatus*, respectively (Bird, Bonnefoy, & Newman, 2011; Chen et al., 2013). The genes for ferrous iron oxidation were absent in acidophilic microbes probably (1) because of the low abundance of microorganisms responsible for iron oxidation, resulting in the difficulty retrieving relevant genes from the metagenome; and (2) owing to limited knowledge of iron oxidation in microorganisms (Bonnefoy & Holmes, 2012; Chen et al., 2013).

Genes for the redox proteins involved in Fe(II) oxidation in acidophiles (such as outer membrane cytochrome *c* or Cys2, blue copper proteins rusticyanin and Cys1) (Bird et al., 2011; Bonnefoy & Holmes, 2012; Chen et al., 2013; Ilbert & Bonnefoy, 2013) were observed in this study (Fig. 1.6). The results indicated that rusticyanin genes (*rus*) existed in the metagenome,

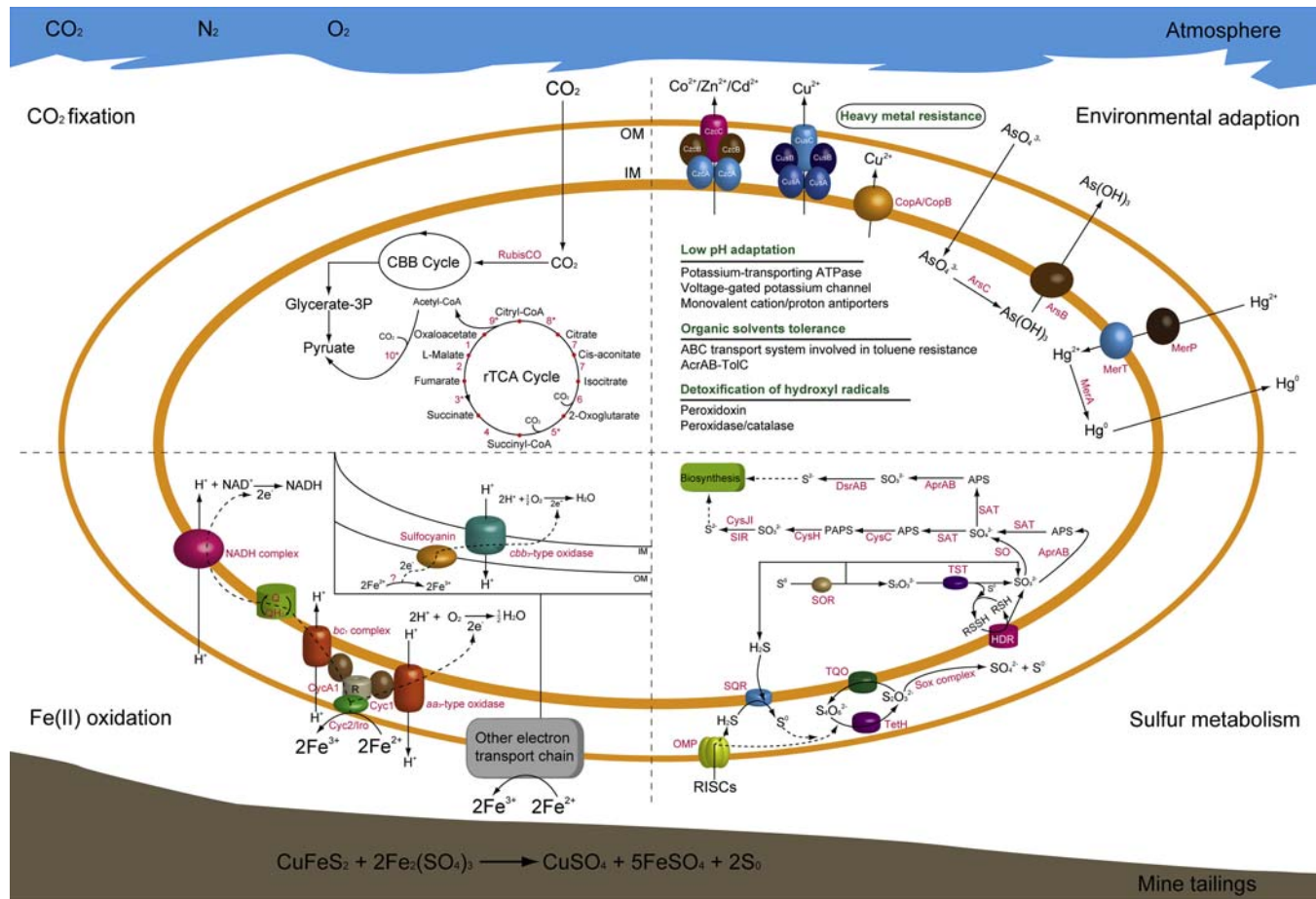


FIGURE 1.6 Overview of main known metabolic abilities (carbon fixation, ferrous iron oxidation, and sulfur metabolism) of microbial community and environmental adaption in surface-layer mine tailings. All possible subsystems are depicted in each quarter section. In CO₂ fixation, enzymes associated with the reductive citric acid (rTCA) cycle are indicated by numbers: 1, malate dehydrogenase; 2, fumarate hydratase; 3, fumarate reductase; 4, succinyl-CoA synthetase; 5, 2-oxoglutarate ferredoxin oxidoreductase; 6, isocitrate dehydrogenase; 7, aconitase hydratase 1; 8, citryl-CoA synthetase; 9, citryl-CoA lyase; and 10, pyruvate ferredoxin oxidoreductase. Enzymes related to nitrogen metabolism, ferrous iron oxidation and sulfur metabolism as abbreviated forms are depicted. APR, adenylsulfate reductase; CBB, CalvinBensonBassham; CysC, adenylsulfate kinase; CysH, phosphoadenosine phosphosulfate reductase; CysJl, sulfite reductase (NADPH) flavoprotein; DsrAB, sulfite reductase; HDR, heterodisulfide reductase; R, Rusticyanin; SAT, sulfate adenyltransferase; SIR, sulfite reductase (ferredoxin); SO, sulfite oxidase; SOR, sulfur oxygenase reductase; SOX, sulfur oxidizing protein; SQR, sulfide quinone reductase; TetH, tetrathionate hydrolase; TQO, thiosulfate:quinone oxidoreductase; TST, thiosulfate sulfurtransferase. This figure was adapted from the previous models (Hua et al., 2015; Levicán, Ugalde, Ehrenfeld, Maass, & Parada, 2008; Méndez-García et al., 2015; Yin et al., 2014a). From Zhang, X., Niu, J., Liang, Y., Liu, X., Yin, H. (2016). Metagenome-scale analysis yields insights into the structure and function of microbial communities in a copper bioleaching heap. BMC Genetics, 17(1), 21. <https://doi.org/10.1186/s12863-016-0330-4>.

most of which were assigned to *A. ferrooxidans*-like species. Moreover, they exhibited genes encoding iron oxidase in *A. ferrivorans*-like, although their number was not enriched. Thus, there were at least two different pathways for ferrous iron oxidation in the iron-oxidizing *Acidithiobacillus* spp.: (1) via rusticyanin, and (2) via high-potential iron sulfur protein encoded by the gene *iro* (Bonnefoy & Holmes, 2012). In acidophilic Archaea, Fe(II) oxidation pathways were also proposed (Bonnefoy & Holmes, 2012; Castelle et al., 2015; Méndez-García et al., 2015), indicating that electrons from Fe(II) oxidation by unknown mechanisms were transferred via sulfocyanin to a *cbh₃*-type terminal oxidase (Fig. 1.6). In the tailings sample, only one sulfocyanin gene in the Archaean genus *Ferroplasma*-like was identified. Given the low relative abundance of Archaea in the surface layer of mine tailings, it was supposed that Archaea might have little role in Fe(II) oxidation within the mine tailings.

1.2.3.4 Sulfur metabolism

A number of known enzymes are related to the sulfur metabolism by microorganisms (Dopson & Johnson, 2012; Johnson & Hallberg, 2008; Yin et al., 2014a). Undoubtedly, the transformation of elemental sulfur with various oxidation states is more complicated than iron metabolism. In the surface layer of the bioleaching heap, genes encoding sulfur metabolic enzymes have not yet been elaborated. In this study, a total of 2479 CDSs were found to be linked to sulfur metabolism, mapping to 48 diverse genes. Sulfur and reduced inorganic sulfur compounds (RISCs) might accumulate in areas where pyrite and other types of sulfide minerals were oxidized by Fe(III) ion (Schippers & Sand, 1999). Thus, the energetically favorable substrates could be used by acidophilic sulfur-oxidizing bacteria (Dopson & Johnson, 2012; Johnson & Hallberg, 2008). Microbial oxidation of sulfur or sulfur compounds requires different enzymatic machineries, including sulfide quinone reductase (SQR), thiosulfate:quinone oxidoreductase (TQO), and tetrathionate hydrolase (TetH) (Fig. 1.6).

As an intermediate form during RISC oxidation, sulfide was oxidized by SQR, the coding genes of which were enriched in the metagenome and were most closely identified as those of *Acidithiobacillus*-like populations, to generate elemental sulfur. In addition, the enriched genes encoding TQO, which catalyzed thiosulfate to produce tetrathionate, were also closely related to *Acidithiobacillus*-like genus. As the substrate of TetH, tetrathionate could be further hydrolyzed to thiosulfate, sulfate, and elemental sulfur (Yin et al., 2014a). The genes encoding the truncated sulfur oxidation protein (Sox) system (*SoxAX*, *SoxB*, and *SoxYZ*) were identified in *Acidithiobacillus*-like and *Thiobacillus*-like microorganisms. Although not enriched, *SoxCD* genes were also detected in the metagenome and identified in *Rhodanobacter*-like and *Thiomonas*-like populations. Sulfur oxygenase reductase (SOR) reported previously in certain bacteria such as *Acidithiobacillus thiooxidans* and *A. caldus* (Chen et al., 2012; Yin et al., 2014a) and thermo-acidophilic Archaea such as *Acidianus tengchongensis* and *Acidianus ambivalens* (Li et al., 2008; Urich et al., 2004) were found in the metagenome. In addition, the genes encoding heterodisulfide reductase (*hdrABC*) and thiosulfate sulfurtransferase (*tst*), which participated in the catalysis of thiosulfate to sulfite, were abundant in *Acidithiobacillus*-like populations. Sulfite would be oxidized directly by sulfite oxidase to sulfate or be catalyzed by adenylylsulfate (APS) reductase and sulfate adenylyltransferase (SAT) to generate sulfate via the reversed dissimilatory sulfate reduction. Remarkably, the *aprAB* genes encoding APS reductase were enriched in the metagenome, most of which were assigned to those of *Thiobacillus*-like populations. No such gene, however, was found in *Acidithiobacillus*-like

populations (Chen et al., 2012; Quatrini et al., 2009; Yin et al., 2014a). Indeed, previous reports showed that *Thiobacillus* species were widely found as the dominant member in some mining environments (Schippers et al., 2010).

Reductive pathways of sulfate, which contained assimilatory sulfate reduction and dissimilatory sulfate reduction, ended with the formation of sulfide, and then entered into the biosynthesis of vital compounds. The coding genes for key enzymes of these processes were most closely related to *Acidithiobacillus*-like and *Thiobacillus*-like populations.

1.2.4 Conclusions

The properties of environments, especially extremely acidic, oligotrophic and heavy metals containing bioleaching heap discussed in this study, shape the microbial community composition and function. Metabolic activities occur in the microbial community, taking on the role of the recycler of substance circulation in the bioleaching system and even in nature. If environmental microorganisms harbor a suit of genes involving response mechanisms, it is probably as a determinant factor to adapt to particular environmental conditions.

1.3 Functional role of coexisting bacteria in bioleaching systems

Acidophilic microorganisms are widely distributed in both pristine environments (e.g., acid rock drainage and volcanic, geothermal areas) and acidic environments of anthropogenic origin (e.g., AMD and bioleaching heaps) (Baker & Banfield, 2003; Bonnefoy & Holmes, 2012; Deneff, Mueller, & Banfield, 2010; Zhang et al., 2016c; Zhang, Niu, Liang, Liu, & Yin, 2016d). Many acidophiles that are metabolically active in anthropogenic environments are obligate chemolithoautotrophs capable of assimilating atmospheric CO₂ and deriving energy from the aerobic oxidation of ferrous iron and/or a variety of sulfur species (Hallberg & Johnson, 2001; Johnson & Hallberg, 2008; Rawlings, 2005). In addition, heterotrophs that assimilate organic carbon and mixotrophs that use both organic and inorganic forms of carbon are found in these acidic settings (Johnson & Hallberg, 2003).

Until recently, much evidence had suggested that *Acidithiobacillus* and *Leptospirillum* were considered the common inhabitants of acidophilic, metal-tolerant microbial consortia in many sulfide-rich mining environments (Breuker, Blazejak, Bosecker, & Schippers, 2009; Chen et al., 2015; Kock & Schippers, 2006, 2008; Xiao et al., 2016; Zhang, Niu, Liang, Liu, & Yin, 2016d). Species of *Acidithiobacillus* isolates were demonstrated iron- and/or sulfur-oxidizing acidophiles phylogenetically affiliated with the class *Acidithiobacillia* (Williams and Kelly, 2013), which mainly includes *A. ferrooxidans* (Valdés et al., 2008a), *A. thiooxidans* (Yin et al., 2014a), *A. caldus* (Valdes et al., 2009), and *A. ferrivorans* (Liljeqvist, Valdes, Holmes, & Dopson, 2011). Four recognized members of iron-oxidizing *Leptospirillum* bacteria (Zhang et al., 2016c), including Group I (*L. ferrooxidans*) (Fujimura et al., 2012), Group II (*Leptospirillum rubrum*, and *L. ferriphilum*) (Jiang et al., 2015), Group III (*Leptospirillum ferrodiazotrophum*), and Group IV (Goltsman et al., 2013), have been documented. In contrast to these obligate chemolithoautotrophs, heterotrophic and/or mixotrophic acidophiles such as *Sulfobacillus* spp. have been also characterized from these environments (Guo et al., 2014; Justice et al., 2014). Despite the relatively poor understanding of the moderately thermophilic *Sulfobacillus*, it was believed to have an important role in the biogeochemical cycle of sulfur (Justice et al., 2014). Several isolated *Sulfobacillus* species, including *Sulfobacillus benefaciens*

(Johnson, Joulain, D'Hugues, & Hallberg, 2008), *S. thermosulfidooxidans* (Justice et al., 2014), *Sulfobacillus sibiricus* (Melamud et al., 2003), and *Sulfobacillus thermotolerans* (Bogdanova et al., 2006), have been used to demonstrate their key metabolic features.

Interactions occur ubiquitously among coexisting microbes in laboratory cultures and natural environments (Gupta & Schuster, 2013; Li & Gu, 2007; Summers et al., 2010; Wintermute & Silver, 2010; Yin et al., 2015). A striking example of an interspecies interaction was found between *Arthrobacter* sp. and *Sphingopyxis* sp. in a coculture (Liang et al., 2017) where Mn(II)-oxidizing activity was presumed to be triggered by contact-dependent interactions of two investigated bacteria, although neither of had the ability to oxidize Mn(II). As in other environments, microbial interactions in mixed communities of acidic environments were described (Baker & Banfield, 2003; Johnson, 1998). The spatiotemporal distribution of different populations might be correlated with differences in environmental conditions of individual ecological niches, and in nutrients or other resources that they could use (Yelton et al., 2013). Thus, it is important to investigate how these physiologically different acidophilic microorganisms interact, thus allowing them to coexist.

In this section, we present the detailed analyses of gene repertoire, metabolic features, and potential functional roles of three acidophilic species, including *A. caldus*, *S. thermosulfidooxidans*, and *L. ferriphilum*, which were isolated from disparate bioleaching heaps located in Dexing Copper Mine (Jiangxi Province, China) and Zijinshan Copper Mine (Fujian Province, China), respectively. A comparative survey based on the bacterial genomes was performed to delineate the genomic and functional differences among these co-occurring acidophiles that potentially contribute to a mutualistic relationship rather than competitive exclusion.

1.3.1 Phylogeny of newly sequenced strains and overall genome statistics

Two novel strains in this study were isolated from Dexing Copper Mine and Zijinshan Copper Mine. The genomes of these two bacterial strains were sequenced, and their 16S rRNA genes were then used for phylogenetic study. Results showed that the novel strains were clustered on a distinct branch within *Leptospirillum* sp. Group II, but they were obviously distinguished from other *Leptospirillum* groups, suggesting a close relationship between new isolates and *Leptospirillum* sp. Group II (Fig. 1.7).

Accordingly, eight *Leptospirillum* genomes available in the GenBank database were chosen for phylogenomic analysis (Table 1.2). The phylogenetic relation among all putative or recognized *Leptospirillum* strains was inferred by comparing the average nucleotide identity (ANI) based on BLAST (ANiB) and tetranucleotide frequency correlation coefficient (TETRA). The values of ANiB ($\geq 97.96\%$) and TETRA (≥ 0.999) that were evaluated by JSpecies strongly indicated that strains DX and ZJ analyzed in our study were closely related to *L. ferriphilum* strains but separate from other *Leptospirillum* spp. (Table 1.2), which further supported the prior notion of 16S rRNA gene-based phylogenetic analysis. In addition, these two newly sequenced strains (*L. ferriphilum* DX and ZJ) shared high values of ANiB (98.56) and TETRA (0.999) (Table 1.2). Collectively, we inferred that novel strains DX and ZJ inspected in this study were phylogenetically affiliated with *L. ferriphilum*.

Genomic features of *L. ferriphilum*, *A. caldus* (Zhang et al., 2016c), and *S. thermosulfidooxidans* (Zhang et al., 2017), the latter two of which were also used as part of this study, are summarized in Table 1.3. Each genome had 20 to 396 contigs with coverage ranging from 52 to 165 $\times$.

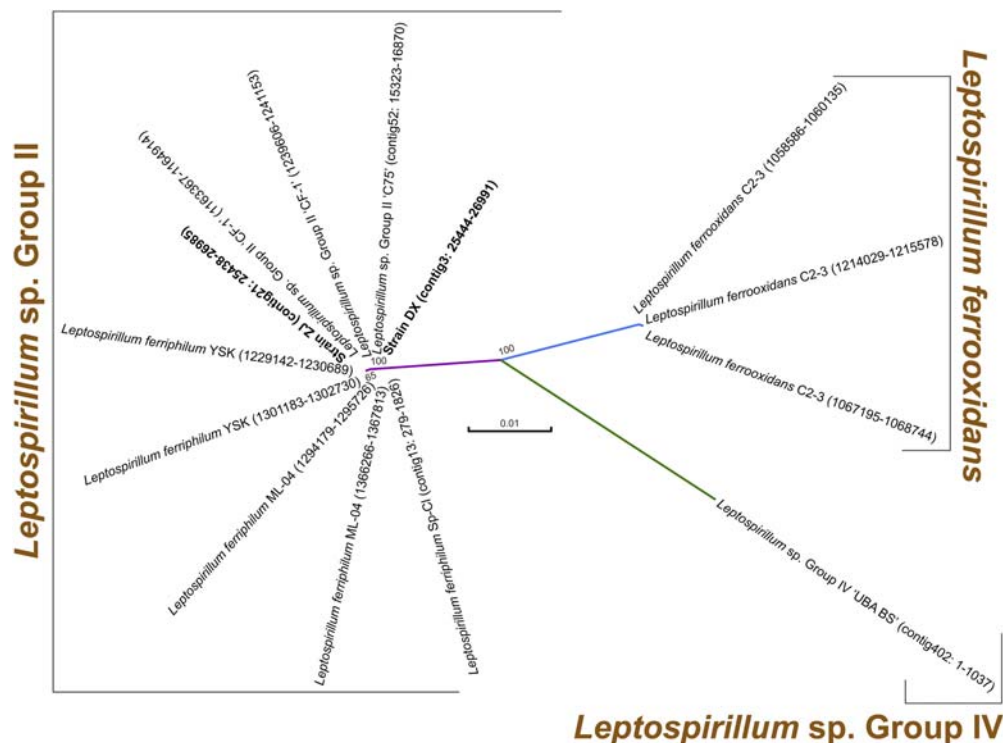


FIGURE 1.7 Phylogenetic tree based on 16S recombinant RNA (rRNA) genes of two newly sequenced strains and other known *Leptospirillum* spp. For each strain, a 16S rRNA gene sequence was extracted from the complete or draft genome using RNAmmer, and its genomic locus was shown. From Zhang X., Liu X., Liang Y., Xiao Y., Ma L., Guo X., Miao B., Liu H., Peng D., Huang W., Yin H. (2017). Comparative genomics unravels the functional roles of co-occurring Acidophilic bacteria in bioleaching heaps. *Frontiers in Microbiology*, 8, 790. <https://doi.org/10.3389/fmicb.2017.00790>.

TABLE 1.2 Genome-based phylogenetic indicators of two novel strains compared with other recognized *Leptospirillum* spp.

Organism	Accession ID	Average nucleotide identity based on BLAST (%) ^a		Tetranucleotide frequency correlation coefficient ^a	
		DX	ZJ	DX	ZJ
Strain DX	MPOJ00000000	—	98.56	—	0.999
Strain ZJ	MP0K00000000	98.56	—	0.999	—
<i>Leptospirillum ferriphilum</i> YSK	CP007243	98.54	98.94	0.999	0.999
<i>L. ferriphilum</i> ML-04	CP002919	98.53	97.97	0.999	0.999
<i>L. ferriphilum</i> Sp-Cl	LGSH01000000	97.96	98.65	0.999	0.999
<i>Leptospirillum</i> sp. Group II 'CF-1'	CP012147	96.80	96.94	0.996	0.996
<i>Leptospirillum</i> sp. Group II 'C75'	AIJM01000000	96.77	96.91	0.999	0.998
<i>Leptospirillum</i> sp. YQP	LIEB00000000	84.42	84.42	0.576	0.578
<i>Leptospirillum</i> sp. Group IV 'UBA BS'	AURA01000000	67.29	66.99	0.870	0.863
<i>Leptospirillum ferrooxidans</i> C2-3	NC_017094	66.63	66.35	0.872	0.874

^aValues below the thresholds of 95% (average nucleotide identity based on BLAST) and 0.99 (tetranucleotide frequency correlation coefficient) suggest that the two strains belong to different species (Richter and Rosselló-Móra, 2009). *Leptospirillum* genus was recognized to harbor four groups, including Group I (*Leptospirillum ferrooxidans*), Group II (*Leptospirillum rubrum*, and *L. ferriphilum*), Group III (*Leptospirillum ferrodiazotrophum*), and Group IV.

Genome completeness assessed by CheckM suggested the near-complete genomes of all six strains, although the genome coverage of *L. ferriphilum* ZJ was relatively low compared with the other strains. *A. caldus* and *S. thermosulfidooxidans* had larger genomes (between 3.12 and 3.18 Mbp) compared with *L. ferriphilum* (between 2.34 and 2.36 Mbp). *A. caldus* strains with higher guanine plus cytosine (GC) contents (DX with 61.01% and ZJ with 61.00%) were observed, whereas the GC contents in *L. ferriphilum* and *S. thermosulfidooxidans* ranged from 48.47% to 54.70%. Compared with the others, GC contents of *A. caldus* genomes were much higher. A plausible explanation for this finding is that the optimal growth temperature is regarded as an environmental factor that positively influences genomic GC content in prokaryotes (Musto et al., 2004, 2006), since *A. caldus* is the primary sulfur oxidizer in bio-leaching operations at temperatures above 40°C (Acuña et al., 2013). Nevertheless, this inference was widely divergent from our recollection, because the moderate thermophile *S. thermosulfidooxidans* harbors relatively low GC contents. In fact, the GC contents of the bacterial genomes varied dramatically, and they were influenced by multiple factors (Hildebrand, Meyer, & Eyre-Walker et al., 2010) such as genome size (Bentley & Parkhill, 2004), environment (Foerstner et al., 2005), nitrogen use (Mcewan et al., 1998), and aerobiosis (Naya et al., 2002). However, more evidence should be provided to determine further whether the relatedness between the genomic GC contents and multifactorial mechanisms is a stochastic process or a result of natural selection.

All of the genome assemblies had the full suite of tRNA that covered all of the 20 amino acids. Bacterial genomes were predicted to contain between 2,342 and 2,958 protein-coding sequences (CDS). The Clusters of Orthologous Groups (COG) annotation suggested that between 75.88% and 83.16% of the CDS were matched to putative proteins with known functions (Table 1.3). Of the 25 COG categories, CDS were assigned to 21 (*L. ferriphilum* and *S. thermosulfidooxidans*) or 22 (*A. caldus*) of them. In addition to COG categories [S] (function unknown) and [R] (general function prediction only), ‘energy production and conversion (C),’ ‘amino acid transport and metabolism (E),’ and ‘cell wall/membrane/envelope biogenesis (M)’ were commonly abundant in all of the genomes (Fig. 1.8). It is important to note that the proportion of CDS assigned to ‘carbohydrate transport and metabolism (G)’ in *S. thermosulfidooxidans* (5.17% in DX and 5.21% in ZJ) was relatively large compared to the other two species (between 2.83% and 3.12%; Fig. 1.8). An analysis of the gene contents showed that all of the genomes were predicted to harbor a large number of CDS that were potentially assigned to the COG categories [C], [E], and [M]. This finding was similar to our previous study (Zhang et al., 2016a). The COG categories [C] and [E] are involved in the metabolic pathways of energy and amino acids, and COG category [M] is related to the synthesis of cell membranes, membrane channel proteins, and extracellular polymeric substances. Thus, we infer that the bacterial lifestyle requires efficient utilization of energy and nutrients from external environments and specialized cellular structures to adapt to changing environments. In addition, the abundant CDS in these bacterial genomes were assigned to COG category [L] (replication, recombination, and repair), which contains many enzymes associated with DNA repair. The habitats that these acidophilic microorganisms inhabit are characterized by extreme acidity and an abundance of heavy metals. When cell damage occurs, the corresponding proteins may play an important role in DNA repair (Chen et al., 2013; Mi et al., 2011). Of note, many CDS assigned to COG category [E] were only identified in *S. thermosulfidooxidans* genomes. We therefore hypothesize that *S. thermosulfidooxidans* strains exhibited additional abilities to synthesize amino acids, suggesting that they depend more heavily on organic compound assimilation than the others.

TABLE 1.3 Comparison of genomic and phenotypic features of co-occurring bacteria isolated from two distinctive copper mines.

Organism	<i>Leptospirillum ferriphilum</i> DX	<i>Acidithiobacillus calvus</i> DX	<i>Sulfobacillus thermosulfidooxidans</i> DX	<i>Leptospirillum ferriphilum</i> ZJ	<i>Acidithiobacillus calvus</i> ZJ	<i>Sulfobacillus thermosulfidooxidans</i> ZJ
Geographic origin	Copper mine tailings, Jiangxi, China			Copper mine tailings, Fujian, China		
Nutritional type	Chemoautotrophic	Chemoautotrophic	Mixotrophic	Chemoautotrophic	Chemoautotrophic	Mixotrophic
Genome status	Draft	Draft	Draft	Draft	Draft	Draft
Accession number	MPOJ000000000	LZYE000000000	MDZD000000000	MPOK000000000	LZYG000000000	MDZF000000000
Coverage	165×	95×	132×	52×	76×	136×
Completeness (%)	93.15	98.76	99.00	93.02	98.14	99.00
Genome size (megabases)	2.36	3.12	3.18	2.34	3.14	3.18
Number of contigs	30	390	33	104	386	20
Guanine plus cytosine content (%)	54.51	61.01	48.47	54.70	61.00	48.47
N50 length (base pairs)	118,116	22,157	171,016	54,724	18,308	452,891
N90 length (base pairs)	51,004	4,321	52,886	12,896	2291	108,775
<i>RNA genes</i>						
5S rRNA count	1	1	1	1	1	2
16S rRNA count	1	1	1	1	1	1
23S rRNA count	1	1	1	1	1	1
tRNA count	46	46	51	46	46	51
Putative CDS	2342	2841	2958	2355	2864	2958
COG	1777 (75.88%)	2250 (79.20%)	2454 (82.96%)	1790 (76.01%)	2274 (79.40%)	2460 (83.16%)

rRNA, recombinant RNA.

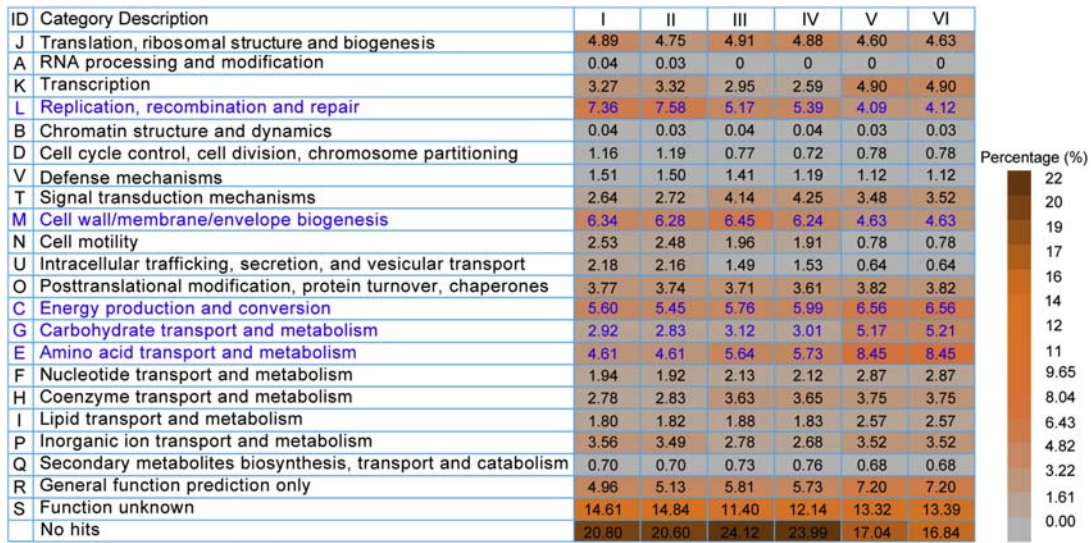


FIGURE 1.8 Heat map depicting percentages of genes assigned to the Clusters of Orthologous Groups classification in *Acidithiobacillus caldus*. DX (I), *A. caldus* ZJ (II), *Leptospirillum ferroplasma* DX (III), *L. ferroplasma* ZJ (IV), *Sulfobacillus thermosulfidooxidans* DX (V), and *S. thermosulfidooxidans* ZJ (VI). The five abundant functional categories within all bacterial genomes are highlighted in blue. From Zhang X., Liu X., Liang Y., Xiao Y., Ma L., Guo X., Miao B., Liu H., Peng D., Huang W., Yin H. (2017). Comparative genomics unravels the functional roles of co-occurring Acidophilic bacteria in bioleaching heaps. *Frontiers in Microbiology*, 8, 790. <https://doi.org/10.3389/fmicb.2017.00790>.

1.3.2 Identification of core genes and flexible genes

There were 8114 CDS clustered using the PanOCT with a 30% sequence identity cutoff to infer shared and flexible genes among the tested strains. Comparative analysis showed that 419 putative orthologous genes were shared by all strains (Fig. 1.9), representing low proportions compared with the entire CDS of the individual genome: 17.89% in *L. ferroplasma* DX, 17.79% in *L. ferroplasma* ZJ, 14.75% in *A. caldus* DX, 14.63% in *A. caldus* ZJ, 14.16% in *S. thermosulfidooxidans* DX, and 14.16% in *S. thermosulfidooxidans* ZJ. These shared genes were mainly associated with translation, ribosomal structure, and biogenesis (J), amino acid transport and metabolism (E), energy production and conversion (C), and nucleotide transport and metabolism (F). Aside from core genes common in all of the strains, the flexible genes, including genes present in some but not all of the genomes and strain-specific genes unique to the individual genomes, are depicted (Fig. 1.9). Notably, the CDS only shared by each species (1302 in *L. ferroplasma*, 2023 in *A. caldus*, and 2262 in *S. thermosulfidooxidans*) were much more than the core genes, clearly suggesting the genomic difference among these organisms. Of these sequences, the COG classification revealed that many were annotated as hypothetical proteins or proteins with unknown functions. In addition, the CDS involved in the COG categories [M] (cell wall/membrane/envelope biogenesis) and [C] were numerically significant, highlighting distinguishing features among these species. Similar to the results shown in Fig. 1.8, the percentage of CDS related to the COG category [G] in the *S. thermosulfidooxidans* species (5.13%) was larger than that in the others (2.53% in *L. ferroplasma* and 1.93% in *A. caldus*). Of genes unique in *S. thermosulfidooxidans*, many were involved in the biosynthesis of amino acid, such as cysteine, arginine, glycine, as well as alanine (unpublished). The number of CDS associated with carbohydrate transport and

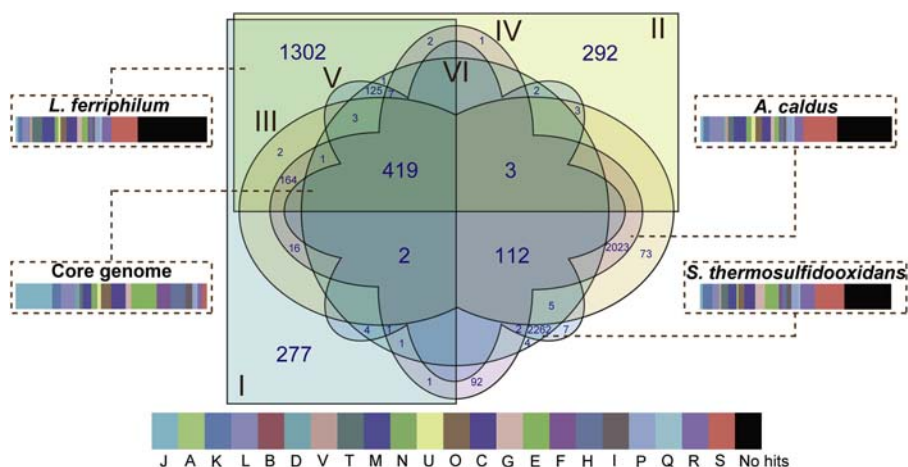


FIGURE 1.9 Venn diagram depicting orthologous and nonorthologous genes. *Leptospirillum ferriphilum* DX (I), *L. ferriphilum* ZJ (II), *Acidithiobacillus caldus* DX (III), *A. caldus* ZJ (IV), *Sulfobacillus thermosulfidooxidans* DX (V), and *S. thermosulfidooxidans* ZJ (VI) are shown in different colors. Numbers shown in the Venn diagram indicate the number of orthologous or strain-specific genes. If no number is given in certain patterns, it suggests there is no orthologous protein among these strains. The core genome and unique genes in each species were used for functional analysis based on Clusters of Orthologous Groups (COG) classification. Descriptions of COG categories are provided in Fig. S1. The width of the rectangle indicates the percentage of CDS compared with the entire genome of individual strains. From Zhang X., Liu X., Liang Y., Xiao Y., Ma L., Guo X., Miao B., Liu H., Peng D., Huang W., Yin H. (2017). Comparative genomics unravels the functional roles of co-occurring Acidophilic bacteria in bioleaching heaps. *Frontiers in Microbiology*, 8, 790. <https://doi.org/10.3389/fmicb.2017.00790>.

metabolism in *S. thermosulfidooxidans* was relatively large compared with its counterparts. Hence, we interpreted this to indicate that the *S. thermosulfidooxidans* strains were mixotrophic acidophiles capable of assimilating inorganic and organic carbon. Collectively, the findings presented here imply that genomic and functional differentiation of these acidophilic species in the same community might allow them to coexist.

BLASTN-based whole genome comparisons of these targeted strains isolated from distinct areas were performed and visualized using Circos (Fig. 1.10) to acquire some insights into intraspecies differences. An examination of the architecture and global structural genomes revealed that a number of nonshared genomic regions were found between strains belonging to the same species, although they shared a high-sequence identity. Further inspection revealed that many genes distributed in the genomic regions (Sections 1–10 in Fig. 1.10) of each *L. ferriphilum* strain were predicted to encode putative proteins with unclear functions.

1.3.3 Comparison of inferred metabolic profiles

The KAAS online server was employed to investigate the metabolic potential of the microorganisms in this study (Table 1.4). In general, four types of metabolism with the most abundant genes in all of the strains were carbohydrate metabolism, amino acid metabolism, energy metabolism, and metabolism of cofactors and vitamins. Metabolism-related CDS in *S. thermosulfidooxidans* with a larger genome (1124 in DX and 1125 in ZJ) were more than that in the other two species (between 818 and 885). Furthermore, the key metabolisms of each genome are listed and comparisons are presented in Table 1.5. In the following section, we discuss the metabolic differences among species.

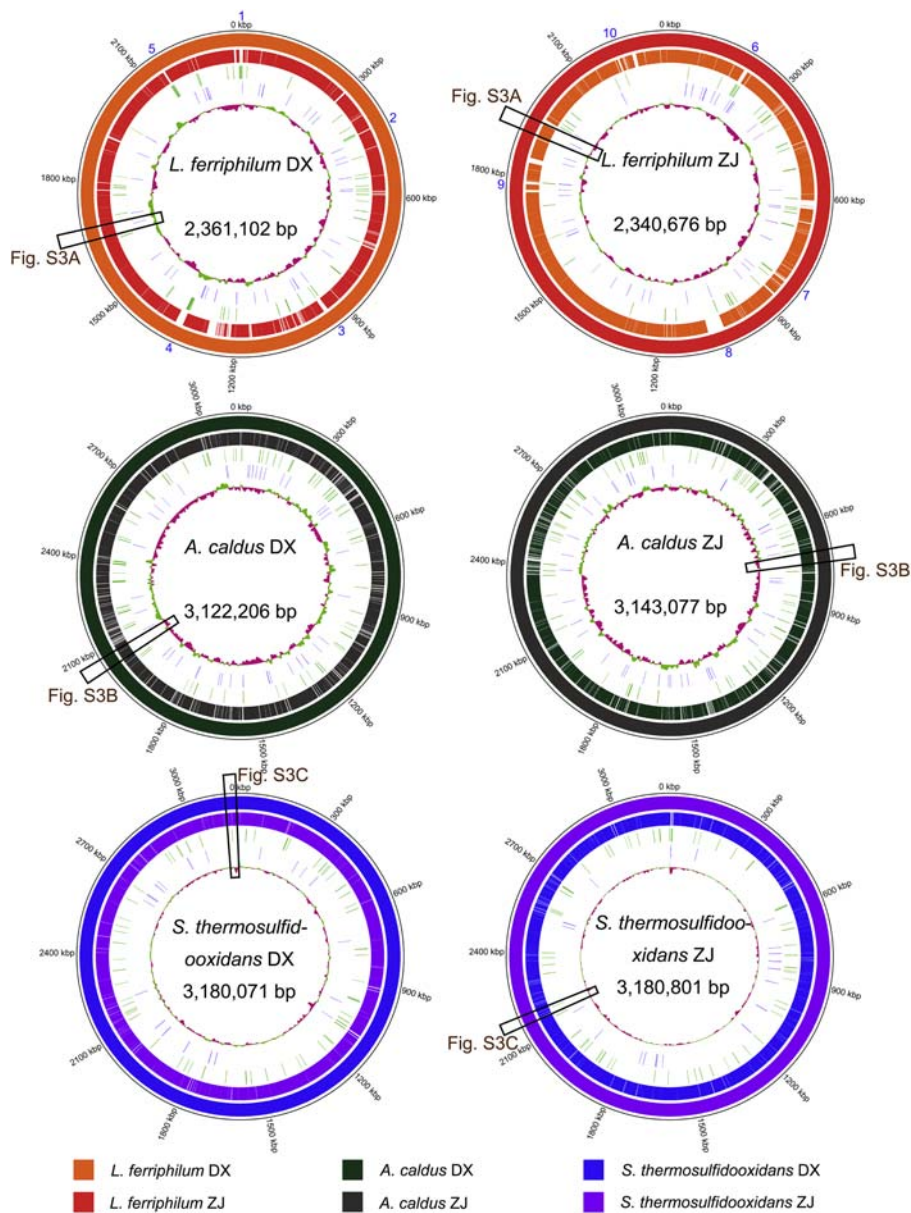


FIGURE 1.10 Reference-based whole genome comparisons and visualization of each strain. Guanine plus cytosine contents of individual genomes are indicated in the center of the figure. In addition, transfer RNA and transposases are shown on the second and the third rings from the inside. Matches to the reference genomes (≥50% sequence identity) are shown with different colors. Cross-references showing further analysis of certain genomic regions are linked to other figures. From Zhang X., Liu X., Liang Y., Xiao Y., Ma L., Guo X., Miao B., Liu H., Peng D., Huang W., Yin H. (2017). Comparative genomics unravels the functional roles of co-occurring Acidophilic bacteria in bioleaching heaps. *Frontiers in Microbiology*, 8, 790. <https://doi.org/10.3389/fmicb.2017.00790>.

TABLE 1.4 Comparisons of inferred metabolic profiles of bacterial genomes.

Pathway	Number of coding sequences					
	<i>Leptospirillum ferriphilum</i>		<i>Acidithiobacillus caldus</i>		<i>Sulfobacillus thermo- sulfidooxidans</i>	
	DX	ZJ	DX	ZJ	DX	ZJ
Carbohydrate metabolism	209	200	198	200	256	257
Energy metabolism	113	116	140	138	168	168
Lipid metabolism	35	32	37	37	44	44
Nucleotide metabolism	76	76	90	89	99	99
Amino acid metabolism	144	141	156	159	244	244
Metabolism of other amino acids	25	24	29	29	53	53
Glycan biosynthesis and metabolism	40	40	44	43	24	24
Metabolism of cofactors and vitamins	109	110	109	110	139	139
Metabolism of terpenoids and polyketides	27	25	29	29	29	29
Biosynthesis of other secondary metabolites	26	26	36	36	36	36
Xenobiotic biodegradation and metabolism	28	28	14	15	32	32

The four most abundant metabolism-related genes are highlighted in bold.

TABLE 1.5 Overall comparisons of sequenced genomes of *L. ferriphilum* DX (1), *L. ferriphilum* ZJ (2), *A. caldus* DX (3), *A. caldus* ZJ (4), *S. thermosulfidooxidans* DX (5), and *S. thermosulfidooxidans* ZJ (6) with reference to key metabolic potentials.

Metabolic pathways	1	2	3	4	5	6
Carbon assimilation						
Calvin cycle						
RuBisCO			2	2	1	1
RubisCO-like protein	1	1	1	1		
Carboxysome						
TCA cycle	i	i	i	i		
rTCA cycle						
Glycolysis / Gluconeogenesis						
Glycolic acid assimilation						
Nitrogen uptake						
Ammonium uptake						
Dissimilatory nitrate reduction						
Assimilatory nitrate reduction						
Urease complex						
Sulfur metabolism						
Sulfur oxygenase reductase			1	1	2	2
Sox system			2	2		
Iron oxidation						
Cyc ₅₇₂						
Cyc ₅₇₉	2	2				
cbb ₃ -type cytochrome oxidase						
bc ₁ complex	2	2				
Sulfocyanin					2	2
aa ₃ -type cytochrome oxidase						

White cells and blue cells represent absence and presence of the metabolic enzymes or metabolic pathways in individual genomes, respectively. Additionally, the number of copies of gene/operon was highlighted in white letterings; *i*, incomplete pathway; *Rubisco*, ribulose-1,5-bisphosphate carboxylase/oxygenase.

1.3.3.1 Central carbon metabolism

As previously noted by Berg et al. (2010), six autotrophic carbon fixation mechanisms that assimilate CO₂ into cellular material have been well-documented, including the CBB cycle, the reductive tricarboxylic acid cycle, the reductive acetyl-coenzyme A pathway, the 3-hydroxypropionate bicycle, the hydroxypropionate-hydroxybutyrate cycle, and the dicarboxylate-hydroxybutyrate cycle. Rubisco, one of the key enzymes associated with the

first rate-limiting step for the CBB cycle, has been classified into forms I–IV (Zhang et al., 2016c). In previous studies, the CBB cycle was considered to be the way to fix carbon in *Lep-tospirillum* genus (Coram & Rawlings, 2002). The current results showed that no gene encoding putative Rubisco was identified in *L. ferriphilum* isolates, but noncanonical Rubisco-like protein was predicted. However, *L. ferriphilum* genomes were predicted to harbor a full suite of genes encoding canonical enzymes, which were involved in the rTCA cycle for carbon fixation. Similar results could be also found in several other reviews and papers (Levicán et al., 2008; Mi et al., 2011; Zhang et al., 2016c). In particular, genes encoding pyruvate ferredoxin oxidoreductase and 2-oxoglutarate ferredoxin oxidoreductase were predicted to exist in a common genome region (Fig. 1.11A). In contrast, *A. caldus* strains were reported to harbor distinct forms of Rubisco, and *S. thermosulfidooxidans* had only type I Rubisco. For the *A. caldus* genomes, genes encoding type I Rubisco were located in a gene cluster involved in carboxysome formation (Fig. 1.11B). Notably, *A. caldus* isolates were predicted to harbor a carboxysome-associated carbonic anhydrase; however, no genome region related to carboxysome was identified in *S. thermosulfidooxidans*, despite the existence of a gene encoding the putative carbonic anhydrase.

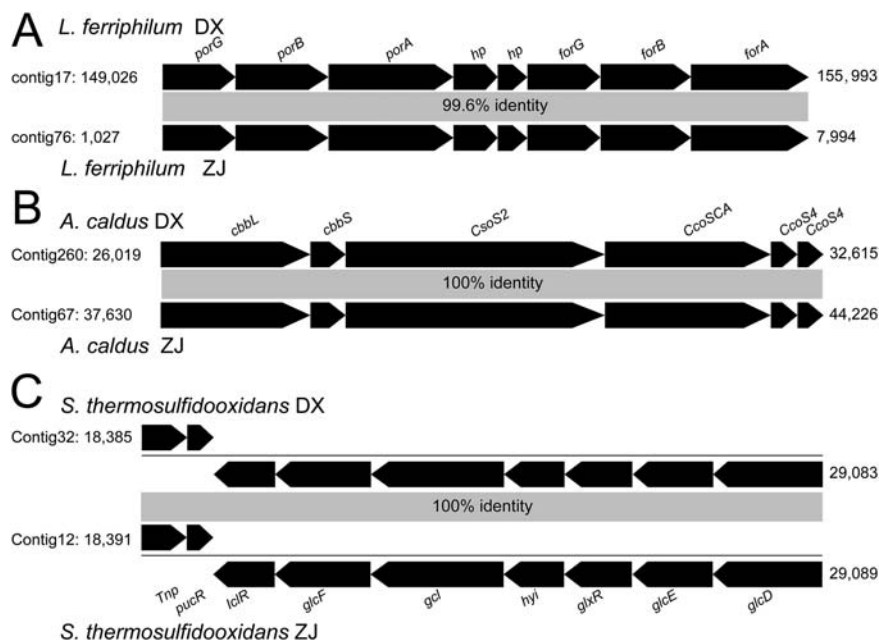


FIGURE 1.11 Homologous genome regions in individual strains associated with (A) pyruvate ferredoxin oxidoreductase and 2-oxoglutarate ferredoxin oxidoreductase, (B) carboxysome, and (C) glycolate oxidase. *cbbL*, Rubisco large chain; *cbbS*, Rubisco small chain; *CsoS2*, carboxysome shell protein *CsoS2*; *CsoS4*, carboxysome shell protein *CsoS4*; *CsoSCA*, carboxysome-associated carbonic anhydrase; *forA*, 2-oxoglutarate ferredoxin oxidoreductase alpha subunit; *forB*, 2-oxoglutarate ferredoxin oxidoreductase beta subunit; *forG*, 2-oxoglutarate ferredoxin oxidoreductase gamma subunit; *gcl*, glyoxylate carboligase; *glcD*, glycolate oxidase subunit *GlcD*; *glcE*, glycolate oxidase FAD binding subunit; *glcF*, glycolate oxidase iron-sulfur subunit; *glxR*, 2-hydroxy-3-oxopropionate reductase; *hp*, hypothetical protein; *hyi*, hydroxypyruvate isomerase; *lclR*, *lclR* family transcriptional regulator; *porA*, pyruvate ferredoxin oxidoreductase alpha subunit; *porB*, pyruvate ferredoxin oxidoreductase beta subunit; *porG*, pyruvate ferredoxin oxidoreductase gamma subunit; *pucR*, PucR family transcriptional regulator; *Tnp*, transposase. From Zhang X., Liu X., Liang Y., Xiao Y., Ma L., Guo X., Miao B., Liu H., Peng D., Huang W., Yin H. (2017). Comparative genomics unravels the functional roles of co-occurring Acidophilic bacteria in bioleaching heaps. *Frontiers in Microbiology*, 8, 790. <https://doi.org/10.3389/fmicb.2017.00790>.

All of the isolates were also anticipated to share a variety of genes predicted to be involved in the synthesis of lipopolysaccharides, peptidoglycan, and, potentially, exopolysaccharides. In all isolates, accumulated 3-phosphoglycerate from carbon fixation was predicted to be converted via glycolysis or gluconeogenesis to produce the major precursors (glucose-6-phosphate and glucose-1-phosphate) for the biosynthesis of cell envelope polysaccharides. In light of an earlier report using *Ferroplasma* genomes (Ullrich, González et al., 2016), a core set of genes potentially associated with amino sugar and nucleotide sugar metabolism (ko00520) were predicted in all investigated strains. A variety of intermediate metabolites, such as uridine diphosphate (UDP)-glucose, UDP-glucuronate, UDP-galactose, and UDP-N-acetyl-glucosamine, within these metabolic processes might be potential precursors for the biosynthesis of cell envelope polysaccharides in all strains. Several genes potentially involved in the formation of precursors, such as UDP-mannose and UDP-N-acetyl-mannosamine, were identified only in the *S. thermosulfidooxidans* genomes, whereas *L. ferriphilum* and *A. caldus* were found to share the potential to produce alternative precursors, such as ADP-glucose and UDP-galacturonate. Furthermore, only the *A. caldus* strains appeared to catalyze guanosine diphosphate (GDP)-mannose to GDP-4-dehydro-6-deoxy-D-mannose by GDP-mannose 4,6-dehydratase (EC 4.2.1.47).

Leptospirillum ferriphilum and *A. caldus* were found to harbor a slightly larger enzyme repertoire related to this biosynthesis, which suggests the production of additional macromolecules with species-specific monosaccharide compositions compared with *S. thermosulfidooxidans*. For mechanisms potentially involved in the export of cell envelope polysaccharides, the ABC-2-type polysaccharide transport system was identified in all strains, whereas the Lpt-type lipopolysaccharide export system was found solely in *L. ferriphilum* and *A. caldus*. Compared to the other microorganisms (Craig, Cherry, & Brady, 2011; Haft et al., 2006; Ullrich, González et al., 2016), only *L. ferriphilum* strains shared the genes predicted to encode the PEP-CTERM exosortase system that was presumed to be involved in the export of exopolysaccharides during biofilm formation. In several earlier studies, biofilm was proposed to provide a reaction space between the sulfide surfaces and bacterial cells, thereby accelerating sulfide mineral dissolution (González et al., 2013; Rohwerder & Sand, 2007; Watling, 2006).

Acidithiobacillus caldus and *L. ferriphilum* were predicted to exhibit an incomplete tricarboxylic acid cycle (TCA) cycle. However, *S. thermosulfidooxidans* strains harbored a full suite of genes encoding the TCA cycle, which was thought to be essential for heterotrophic growth (Wood, Aurikko, & Kelly, 2004). In addition, *S. thermosulfidooxidans* strains exhibited the potential ability to degrade glycolic acid, because genes encoding putative glycolate oxidase and malate synthase were found in both genomes. In particular, *S. thermosulfidooxidans* strains were predicted to harbor genomic regions potentially associated with glycolate oxidase (Fig. 1.11C). A pairwise comparison of these gene clusters revealed the identical order and orientation of all genes, as well as the nucleotide sequence identities of up to 100%, which suggests that the gene clusters involved in glycolate oxidase might evolve from a common ancestor.

As for comparison and analysis, genome sequences of these six strains were used to identify carbohydrate-active enzymes (CAZymes), which represented an enormous number of enzymes responsible for the assembly, modification, and breakdown of oligo- and polysaccharides (Lombard, Ramulu, Drula, Coutinho, & Henrissat, 2013). The gene repertoire of potentially encoding CAZymes, including glycoside hydrolases (GHs), glycosyltransferases,

polysaccharide lyases, carbohydrate esterases (CEs), auxiliary activities, and carbohydrate-binding modules, were identified in all genomes. Apparently, more abundant CAZymes were identified in the *S. thermosulfidooxidans* genomes (156 in DX and 157 in ZJ) compared with the others (between 80 and 92), especially GHs responsible for the hydrolysis of glycosidic bonds and CEs that hydrolyze carbohydrate esters. The most abundant GH classes in the *S. thermosulfidooxidans* isolates were strongly biased toward the catabolism of oligo- or polysaccharides, such as chitinase (GH18), cellobiohydrolase (GH74), glucoamylase (GH15), and α -amylase (GH13).

Similar to an earlier study (Yelton et al., 2013), another potential organic carbon source for *S. thermosulfidooxidans* strains might be lipids from lysed cells. They were predicted to harbor a full set of putative proteins involved in fatty acid degradation, including long-chain acyl-CoA synthetase, acyl-CoA dehydrogenase, enoyl-CoA hydratase, and acetyl-CoA acyltransferase. In addition, lactate permease and lactate use of protein B/C were identified in *S. thermosulfidooxidans* isolates, which suggests their heterotrophic growth on lactate.

An earlier study revealed that *Acidithiobacillus* spp. and/or *Leptospirillum* spp. were observed to be the major carbon fixers in AMD communities (Chen et al., 2015). Previous studies showed detailed evidence supporting the fixation of carbon dioxide via the classical CBB cycle in *A. caldus* (You et al., 2011; Zhang et al., 2016b) and *S. thermosulfidooxidans* (Guo et al., 2014; Justice et al., 2014; Zhang et al., 2017). Apart from autotrophic growth, *S. thermosulfidooxidans* isolates have been reported to grow heterotrophically via the catabolism of various organic carbon compounds (Guo et al., 2014; Justice et al., 2014). Unlike *S. thermosulfidooxidans*, *A. caldus* was predicted to elevate the concentration of carbon dioxide near Rubisco by a carboxysome-associated carbonic anhydrase, which was responsible for converting accumulated cytosolic bicarbonate into CO₂ (Zhang et al., 2016e). Thus, carboxysome within *A. caldus* strains might be a critical component that contributes to their ability to grow autotrophically through the efficient use of scarce carbon availability from acidic environments.

Certain organic compounds such as cell exudates or lysates that originated from the autotrophic primary producers might be used by heterotrophic prokaryotes (Johnson & Hallberg, 2003). Glycolic acid (C00160) was reported to be an exudate originating from autotrophic acidophiles, such as *Leptospirillum* (Justice et al., 2014). In contrast to *Sulfobacillus acidophilus* (Justice et al., 2014), no genes encoding putative isocitrate lyase (EC 4.1.3.1) were identified in *S. thermosulfidooxidans*. Accordingly, we surmise that *S. thermosulfidooxidans* might not have the ability to assimilate isocitrate to malate via glyoxylate bypass, a pathway closely related to the TCA cycle (Yelton et al., 2013). In addition, a gene cluster involved in glycolate oxidase was predicted in *S. thermosulfidooxidans*. A putative transposase identified in its downstream region suggested that this cluster might be introduced by horizontal gene transfer (HGT), thereby recruiting a novel ability to degrade the organic carbon (Fig. 1.11C). Taken together, we present a potential route of glycolic acid assimilation that enables *S. thermosulfidooxidans* to use organic acid as an alternative carbon source (Fig. 1.12). In this pathway, glycolate oxidase coupled with malate synthase may degrade glycolic acid to produce malate, and the latter then enters the conventional TCA cycle. Chemolithotrophic acidophiles were sensitive to organic acids in their habitats (Johnson, 1998); therefore, it might be favorable for chemoautotrophs to form a mutualistic relation with other heterotrophic and/or mixotrophic acidophiles, because the latter could effectively detoxify the environments by degrading organic compounds (Johnson & Hallberg, 2003).

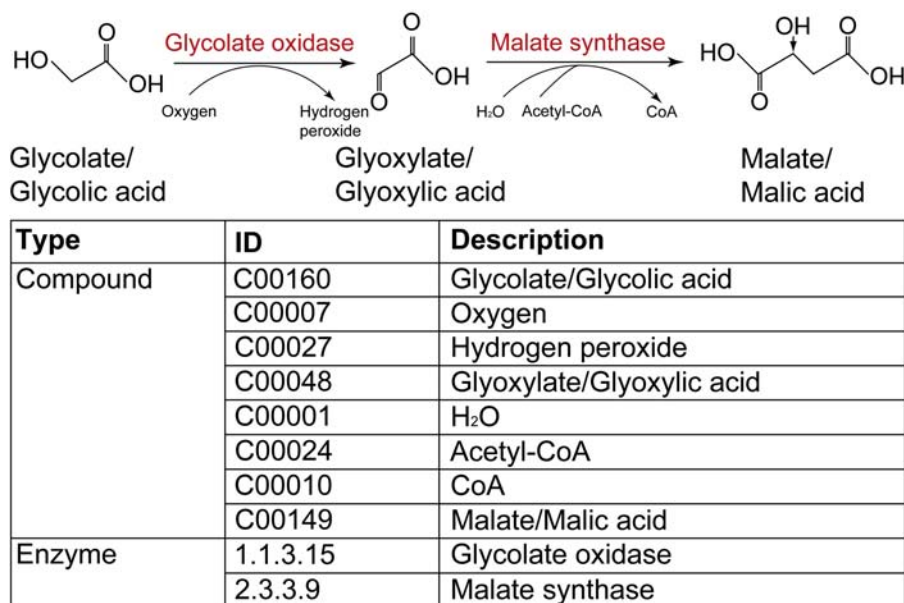


FIGURE 1.12 Putative route for glycolic acid assimilation within *Sulfobacillus thermosulfidooxidans* strains. From Zhang X., Liu X., Liang Y., Xiao Y., Ma L., Guo X., Miao B., Liu H., Peng D., Huang W., Yin H. (2017). Comparative genomics unravels the functional roles of co-occurring Acidophilic bacteria in bioleaching heaps. *Frontiers in Microbiology*, 8, 790. <https://doi.org/10.3389/fmicb.2017.00790>.

In addition, CDS assigned to chitinases (GH18) were found in *S. thermosulfidooxidans*. The GH18 family contained chitinolytic enzymes involved in chitin degradation, and chitin is known to be a substantial component of fungal cell walls. An intriguing explanation for the abundant chitinolytic enzymes in *Trichoderma* is that chitinases are regarded as an integral part of the mycoparasitic lifestyle via lysis of the prey's cell walls (Kubicek et al., 2011). In our study, we could not exclude the possibility that putative chitinases present only in *S. thermosulfidooxidans* might endow them with an additional ability to degrade the lysates of fungi in bioleaching systems. This scenario might be reasonable because fungi were found in several acidic environments, such as AMD ecosystems (Mosier et al., 2016), although their functional roles in bioleaching heaps remain elusive (Cárdenas, Quatrini, & Holmes, 2016).

1.3.3.2 Nitrogen uptake

Acidithiobacillus caldus strains harbor the complete genes required for dissimilatory nitrate reduction, which were observed as *NarGHJI* and *nirBD* operons. Thus, it was hypothesized that the *A. caldus* strains in this study might use nitrate and nitrite as electron acceptors under anaerobic environmental conditions. As for assimilatory nitrate reduction, *A. caldus* was predicted to harbor *narA* potentially as an encoding assimilatory nitrate reductase catalytic subunit; however, there was no NasB subunit, which was reported to transfer electrons from nitrite reductase (NADH) to nitrate (Lin & Stewart, 1997), thus making the electron donor unclear. In addition, no *nirA* gene was identified in the *A. caldus* genomes. In contrast, *S. thermosulfidooxidans* share the set of genes that encode assimilatory nitrate reduction. We identified the genes encoding the assimilatory nitrate reductase catalytic subunit and ferredoxin-nitrite reductase (NirA). Although putative genes potentially encoding

dissimilatory nitrite reductase small subunit and NirA were found in the *L. ferriphilum* genomes, their functional roles need to be studied further. In addition, all of the strains share the potential to use ammonium via the Amt family transporter and to assimilate it into the central metabolic pathways via glutamine synthetase or glutamate synthase. Because dissimilatory nitrate reduction and assimilatory nitrate reduction were absent in *L. ferriphilum*, they might use ammonium as the sole nitrogen source. Moreover, *S. thermosulfidooxidans* was the only species that contains gene cluster encoding urease subunits and accessory proteins. Accordingly, it seems that *S. thermosulfidooxidans* could use extracellular urea, although there is still no direct evidence showing the existence of these compounds in the bioleaching heaps.

Like other microorganisms in AMD that are characterized by scarce nitrogen availability (Chen et al., 2015; Parro, Moreno-Paz, & González-Toril, 2007), the inhabitants in the bioleaching systems may share nitrogen assimilation strategies to cope with nitrogen-limited conditions. In general, microorganisms commonly use atmospheric nitrogen (N₂), ammonium, nitrite, and nitrate as their inorganic nitrogen sources (Arrigo, 2005). As a member of the *Acidithiobacillus* genus, *A. ferrooxidans* has been predicted to harbor nitrogenase, which is responsible for N₂ fixation (Levicán et al., 2008; Zhang et al., 2016e). Likewise, nitrogen fixation was also reported in the *Leptospirillum* groups, except for Group II (Goltsman et al., 2013). Under low nitrogen levels, the diazotrophic lifestyle of these bacteria in the microbial communities might be a striking feature to maintain the concentration of nitrogen availability in the habitat environments.

Although the three species analyzed in this study do not have the ability to fix atmospheric nitrogen, they were predicted to use environmental ammonium, nitrite, and even nitrate as an alternative nitrogen source. *Leptospirillum ferriphilum* was predicted merely to harbor the ability of ammonium uptake, but the other two species could use other forms of nitrogen such as nitrate or nitrite as the alternative nitrogen source, to some extent avoiding competitive exclusion. Notably, microbial ureases have been reported to hydrolyze environmental urea to produce ammonia and bicarbonate (Mobley & Hausinger, 1989; Ullrich, González, et al., 2016; Ullrich, Poehlein, et al., 2016), and ammonia at circumneutral cytoplasmic pH is present as ammonium (Ullrich et al., 2016b). Thus, the *S. thermosulfidooxidans* strains appear to be able to incorporate cytoplasmic ammonium derived from the hydrolysis of urea as a nitrogen source (Fig. 1.13) and use the released bicarbonate as an alternative carbon source via carbonic anhydrase.

1.3.3.3 Iron-sulfur cycling

Both *L. ferriphilum* and *S. thermosulfidooxidans* are demonstrated iron oxidizers (Guo et al., 2014; Justice et al., 2014; Zhang et al., 2016c). These two species were predicted to harbor membrane-associated *c*-type cytochromes that are potentially involved in iron oxidation. In *L. ferriphilum*, the electrons might be transferred from an outer-membrane cytochrome *c* (Cyc₅₇₂) to a periplasmic cytochrome *c* (Cyt₅₇₉), and then across periplasmic cytochromes *c* either reducing oxygen via *cbb*₃-type terminal oxidase (downhill), or reducing NAD⁺ passing through the *bc*₁ complex, quinone pool, and NADH dehydrogenase (uphill (Bonnefoy & Holmes, 2012)). Similar to Cyt₅₇₉ in *L. ferriphilum*, the periplasmic sulfocyanins identified in *S. thermosulfidooxidans* were postulated to be the branch point at which electrons derived from membrane *c*-type cytochromes might be channeled either downhill or uphill. As for the uphill pathway, the *bc*₁ complex in *Leptospirillum* spp. was proposed to use the proton motive force (PMF) to push electrons uphill against the thermodynamically unfavorable

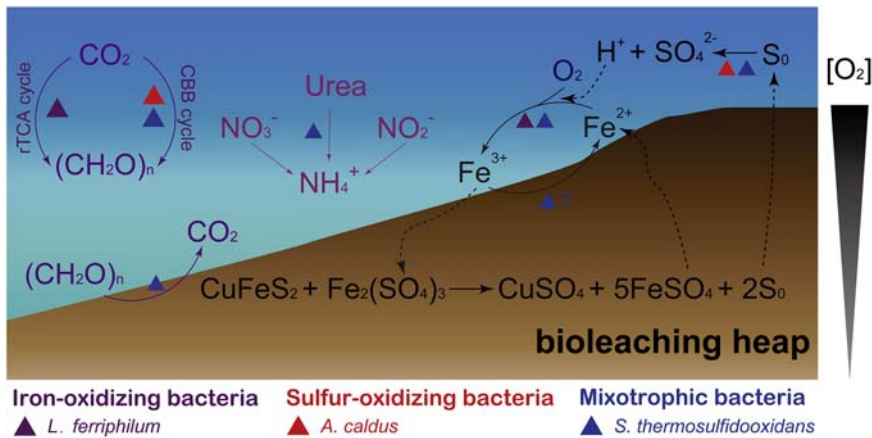


FIGURE 1.13 Schematic representation showing the microbe-mediated biogeochemical cycle of main elements (carbon, nitrogen, iron, and sulfur) in bioleaching heaps. rTCA, reductive citric acid. Adapted from Johnson, D. B. (1998). Biodiversity and ecology of acidophilic microorganisms. *FEMS Microbiology Ecology*, 27(4), 307–317. [https://doi.org/10.1016/S0168-6496\(98\)00079-8](https://doi.org/10.1016/S0168-6496(98)00079-8), Baker, B. J., & Banfield, J. F. (2003). Microbial communities in acid mine drainage. *FEMS Microbiology Ecology*, 44(2), 139–152. [https://doi.org/10.1016/S0168-6496\(03\)00028-X](https://doi.org/10.1016/S0168-6496(03)00028-X), and Chen, L. X., Huang, L. N., Méndez-García, C., Kuang, J. L., Hua, Z. S., Liu, J., et al (2016). Microbial communities, processes and functions in acid mine drainage ecosystems. *Current Opinion in Biotechnology*, 38, 150–158. <https://doi.org/10.1016/j.copbio.2016.01.013>. From Zhang X., Liu X., Liang Y., Xiao Y., Ma L., Guo X., Miao B., Liu H., Peng D., Huang W., Yin H. (2017). Comparative genomics unravels the functional roles of co-occurring Acidophilic bacteria in bioleaching heaps. *Frontiers in Microbiology*, 8, 790. <https://doi.org/10.3389/fmicb.2017.00790>.

gradient (Bonnefoy & Holmes, 2012). Here, the outflux of protons catalyzed by the *cbb*₃-type terminal oxidase contributes to the generation of the PMF during electron transfer to oxygen via a downhill pathway. However, genes encoding the putative *bc*₁ complex were absent in *S. thermosulfidooxidans*. It seems that this species may not require reverse electron transfer to generate reduction equivalents (e.g., NADH and NADPH), but depend on the oxidation of organic compounds, such as the TCA cycle, oxidative pentose phosphate pathway, to produce these compounds, because it was reported to be mixotroph, capable of assimilating both inorganic and organic carbon compounds. In addition, *A. caldus* and *S. thermosulfidooxidans* analyzed here were replete with metabolic enzymes related to sulfur oxidation; they have the ability to oxidize minerals, which produce sulfuric acid (Fig. 1.13). Their activities facilitate the dissolution of base metals in certain acid-soluble sulfide minerals, such as chalcocite, via an oxidative route (Johnson & Hallberg, 2003). Oddly, gene-encoding putative TQO was absent in both *A. caldus* strains, whereas the corresponding gene was identified in the homologous strains such as SM-1 and ATCC 51,756 (Zhang et al., 2016b). In addition, gene-encoding SAT was not found in *S. thermosulfidooxidans* genomes analyzed in this study; however, the homologous strain ST was reported to harbor the *sat* gene (Guo et al., 2014; Zhang et al., 2017). In *A. caldus* (Chen et al., 2012), electrons from TQO, sulfide quinone reductase, sulfur oxidizing protein, and heterodisulfide reductase were mediated by the quinol pool, and then (1) were transferred to oxygen via *bd*-type or *bo*₃-type terminal oxidases, or (2) or were transferred to NADH complex I. Similarly, genes encoding putative *bd*-type terminal oxidase and NADH complex I were identified in *S. thermosulfidooxidans* genomes.

The models for potential iron-sulfur cycling were well-studied in acidic and high-sulfur environments (Baker & Banfield, 2003; Chen et al., 2016; Johnson, 1998; Méndez-García et al., 2015; Zhang et al., 2016e). In these settings, ferrous iron oxidation implemented by iron-oxidizing bacteria, such as *L. ferriphilum* and *S. thermosulfidooxidans*, is the primary biochemical transformation of iron. In addition, the oxidation of sulfide minerals, elemental sulfur, and tetrathionate has been well-documented in *A. caldus* and *S. thermosulfidooxidans* (Chen et al., 2012; Guo et al., 2014; Mangold, Valdés, Holmes, & Dopson, 2011; Zhang et al., 2016b). Fe(III) under anoxic conditions could accept electrons originating from inorganic (e.g., sulfur and/or hydrogen) and/or organic donors (e.g., glucose and/or glycerol), which occurs in the case of chemolithotrophic and heterotrophic acidophiles, respectively (Méndez-García et al., 2015). When exposed to moisture and air, sulfur-containing mineral ores oxidize spontaneously in situations in which molecular oxygen or Fe(III) acts as an oxidant (Johnson & Hallberg, 2003). The formation of acidic environments in turn provides advantages to stabilizing Fe(II), even in the presence of atmospheric oxygen (Bonnefoy & Holmes, 2012); this is an opportunity for iron-oxidizing microorganisms inhabiting these settings to use Fe(II) as a source of energy.

Acidithiobacillus caldus and *S. thermosulfidooxidans* strains used in this study were predicted to lack TQO and SAT encoding genes, respectively. One plausible explanation for this issue was that missing genes might be artifacts of assembly or gene-calling procedure. On the other hand, we could not exclude the possibility that gene *tqo* in homologous strains of *A. caldus* (SM-1 and ATCC 51,756) and *sat* in homologous strains of *S. thermosulfidooxidans* (ST) might be introduced by HGT. With respect to ferric iron [Fe(III)] reduction, previous studies showed that *Sulfobacillus* spp. harbored the ability to reduce ferric iron (Bogdanova et al., 2006; Johnson et al., 2008; Melamud et al., 2003). Although Fe(III) reduction activities under oxygen-limiting conditions were observed in acidophilic microorganisms, the determination of a precise enzymatic system is still an important goal for future studies.

1.3.4 Conclusions

Comparative genomics of three typical co-occurring acidophiles in bioleaching heaps yields valuable insights into the differences in gene content, metabolic profiles, and potential functional roles. In short, it could present a scenario in which certain essential functions are partitioned in co-occurring members of microbial communities, and individual members harboring different metabolic functions generate not merely commensalistic but mutualistic interactions. Thus, it might provide a selective advantage for the efficient use of limited resources in their habitats, in which they benefit from each other to maintain their respective lifestyle.

1.4 Ecology and metabolism of sulfur- and/or iron-oxidizing *Acidithiobacillus*

Over decades, many studies revealed that the acidophilic ferrous and/or sulfur oxidizers, *Acidithiobacillus* spp., were frequently identified in diverse mine tailings worldwide (Bosecker, Mengel-Jung, & Schippers, 2004; Breuker, Blazejak, Bosecker, & Schippers, 2009; Chen et al., 2013; Zhang, Niu, Liang, Liu, & Yin, 2016d). These organisms are responsible for the dissolution of sulfur-bearing minerals by oxidizing dissolved iron(II) ions to iron(III)

ions (Rohwerder, Gehrke, Kinzler, & Sand, 2003) while accelerating metal sulfide oxidation. Moreover, they have a key role in transforming carbon and nitrogen (Falagán, Sánchez-España, & Johnson, 2014) in bioleaching systems and even in natural environments. Although extremely acidic environments challenge the growth of microorganisms in the presence of metal-laden and nutrient-deficient solutions, the members of genus *Acidithiobacillus* are remarkably adaptable owing to their ability to develop specialized mechanisms to cope with extremely harsh conditions (Guazzaroni, Morgante, Mirete, & González-Pastor, 2013; Méndez-García et al., 2015).

Acidithiobacillus genus, the gram-negative, extremely acidophilic, and chemolithoautotrophic γ -proteobacteria, currently contains four recognized species: *A. ferrooxidans*, *A. thiooxidans*, *A. caldus*, and *A. ferrivorans* (Liljeqvist, Valdes, Holmes, & Dopson, 2011; Valdés et al., 2008a; Yin et al., 2014b; You et al., 2011). These autotrophic microorganisms isolated from extremely acidic environments have the ability to acquire energy by oxidizing elemental sulfur and a wide range of RISCs (Hallberg, 2010). In addition, *A. ferrooxidans* and *A. ferrivorans* could use energy and electrons derived from the oxidation of ferrous iron for CO₂ and nitrogen fixation, and other anabolic pathways (Hallberg, 2010; Quatrini et al., 2009).

The availability of genome sequences for these iron and/or sulfur oxidizers belonging to *Acidithiobacillus* is rapidly increasing because of the development of high-throughput sequencing and bioinformatics-based approaches. Since the introduction of high-throughput sequencing technologies dating back to 2008, numerous genomes of *Acidithiobacillus* spp. isolated from acidic environments in various countries have been sequenced and submitted to GenBank. In recent decades, numerous studies dependent on genome sequencing focused on important issues mainly related to key metabolic pathways (Levicán, Ugalde, Ehrenfeld, Maass, & Parada, 2008; Mangold, Valdés, Holmes, & Dopson, 2011; Valdés et al., 2008a; Valdés, Pedroso, Quatrini, & Holmes, 2008b; Yin et al., 2014a), including carbon and nitrogen assimilation, RISCs and iron oxidation, as well as hydrogen and formate use. *Acidithiobacillus ferrooxidans* and *A. ferrivorans* are believed to have key roles in the biogeochemical cycle of iron. In these microbes, the Fe (II) oxidation model, which mainly involves two types of rusticyanins as well as two forms of high-potential iron-sulfur proteins, has been well-studied (Bonnefoy & Holmes, 2012; Talla et al., 2014; Valdés et al., 2008a; Zhang et al., 2016c).

The acquisition of genome sequences provides details for discovering additional genes and further fuels a new field named comparative genomics (Jacobsen et al., 2011). To explore the metabolic diversity in extremely acidic environments in an integrated fashion, basis on previous studies, the genomes of 10 typical acidophiles belonging to four species of *Acidithiobacillus* were selected for a comparative survey (Table 1.6). All bacterial strains used in this section can be withstand a low pH (approximately 2.0) and acquire energy, a carbon source, and a nitrogen source from extremely acidic environments, maintaining their basal metabolic activities. In this section, comparative genomics of 10 bioleaching bacteria would be conducive for revealing and elucidating their metabolic differences.

1.4.1 General characteristics of all four *Acidithiobacillus* species

As reported by Hallberg, González-Toril, & Johnson (2010), 16S rRNA phylogeny together with physiological traits showed evidence that *A. ferrivorans* was identified as a novel species rather than *A. ferrooxidans*. In our study, phylogenomic analysis further revealed that

TABLE 1.6 Summary of *Acidithiobacillus* species and their genomes analyzed in this work.

Species	Strain	Habitat	Temperature (optimum)	pH range	Accession number	Size (Mb)	Status	References
<i>A. caldus</i>	SM-1	Pilot bioleaching reactor	Moderate thermophilic (45°C optimum)	2.0–2.5	NC_015850-54	3.24	Complete	(You et al., 2011)
	ATCC 51756	Coal spoil at Kingsbury mine, United Kingdom			NZ_CP005986-89	2.99	Complete	(Valdes et al., 2009)
<i>A. thiooxidans</i>	A01	Wastewater of coal dump, China	Mesophilic (30°C optimum)	2.0–2.5	AZMO000000000	3.82	Draft	(Yin et al., 2014b)
	ATCC 19377	Kimmeridge clay, United Kingdom			AFOH000000000	3.02	Draft	(Valdes et al., 2011)
	Licanantay	Copper mine, Atacama, Chile			JMEB000000000	3.94	Draft	(Travisanyet al., 2014)
<i>A. ferrivorans</i>	SS3	Norilsk, Russia	Psychrotolerant	1.8–2.0	NC_015942	3.21	Complete	(Liljeqvist et al., 2011)
	CF27	Cobalt/copper mine, Idaho, United States			CCCS000000000	3.43	Draft	(Talla et al., 2014)
<i>A. ferrooxidans</i>	ATCC 53993	NG	Mesophilic (30°C optimum)	1.8–2.0	NC_011206	2.89	Complete	NG
	ATCC 23270	Bituminous effluent of coal mine			NC_011761	2.98	Complete	(Valdés et al., 2008a)
	YQH-1	River in Wudalianchi volcano, China			LJBT000000000	3.11	Draft	(Yan et al. 2015)

NG, not given.

A. ferrivorans could be clearly distinguished from *A. ferrooxidans* (Fig. 1.14A). Pangenome analysis showed a large number of orthologs, their core genome (1102 genes). In addition, a pairwise BLAST comparison illustrated that there were numerous unique genes with strain specificity, probably indicating the hereditary difference at the species and/or strain level (Fig. 1.14B). The unique genes in *A. ferrooxidans* were much lesser than those in other species and/or strains. In *A. ferrivorans* and *A. thiooxidans*, except for *A. thiooxidans* ATCC 19,377, however, strain-specific genes were more numerous, which suggests that the large flexible

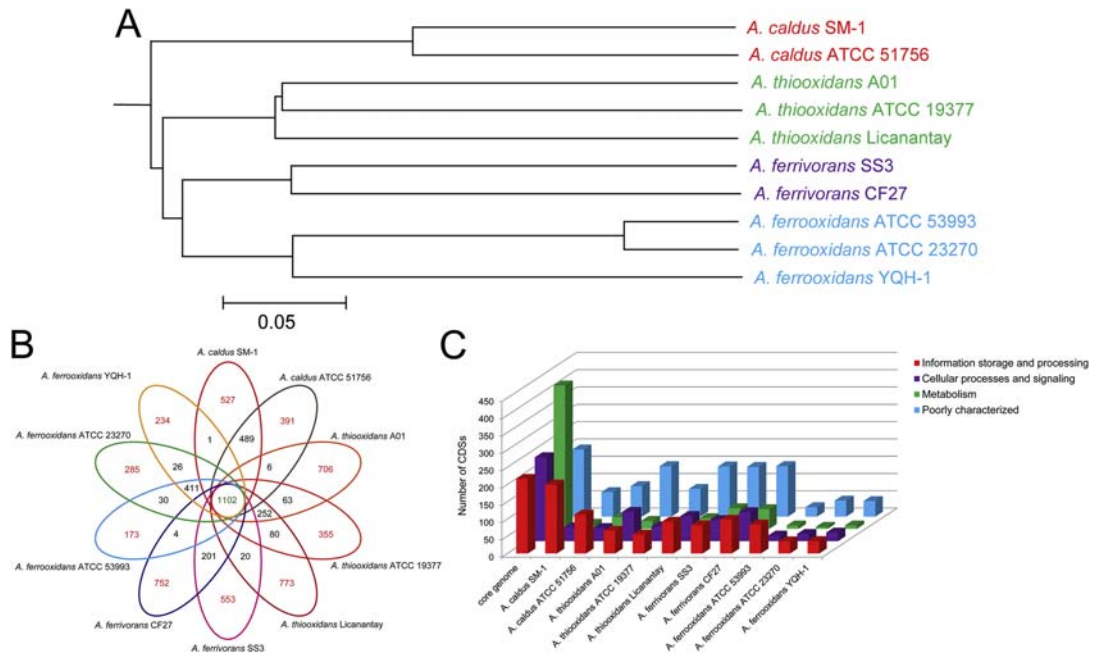


FIGURE 1.14 (A) Phylogenomic tree based on complete or draft genome sequences of *Acidithiobacillus* species and/or strains using the online server CVTree3, and pangenome analyses; (B) Core genome and dispensable/accessory genome of four *Acidithiobacillus* species, including 10 different strains; (C) Clusters of Orthologous Groups catalogs of core genome and strain-specific genes. Numbers in each section represent various contents. The center of the Venn diagram represents the number of orthologous genes shared by all genomes. Each petal represents the number of specific genes in individual strain; other regions show the number of the dispensable or accessory genome. From Zhang X., She S., Dong W., Niu J., Xiao Y., Liang Y., Liu X., Zhang X., Fan F., Yin H. (2016). Comparative genomics unravels metabolic differences at the species and/or strain level and extremely acidic environmental adaptation of ten bacteria belonging to the genus *Acidithiobacillus*. *Systematic and Applied Microbiology*, 39(8), 493–502. <https://doi.org/10.1016/j.syapm.2016.08.007>.

gene pool could confer an advantage to inhabit local environmental conditions. The COG classification further indicated that most of the core genome was assigned to metabolism-related genes, whereas the strain-specific genes represented the large proportion of functional class with poorly characterized properties (Fig. 1.14C). Compared with other strains, *A. ferrivorans* had a much larger number of unique genes mapped to the metabolism-related gene pool. Because *A. ferrivorans* is a psychrotolerant bacterium, it was hypothesized that *A. ferrivorans* might have an advantage in metabolic functions with better adaption to low-temperature environments.

Many similarities regarding environmental features were observed among the bacterial strains analyzed (Table 1.6). Closer inspection revealed several functional differences that reflected their metabolic pathways (Table 1.7). For instance, unlike *A. ferrooxidans* and *A. ferrivorans*, although *A. thiooxidans* and *A. caldus* have no ability to fix nitrogen, they could obtain a nitrogen source in other ways. In addition, the presence or lack of certain key genes such as *sor* and *sir* indicates the difference of sulfur metabolism among these species.

TABLE 1.7 Analysis of key genes/operons or pathways associated with metabolism and environmental adaptation in *Acidithiobacillus* spp.

Function	1*	2*	3*	4*	5*	6*	7*	8*	9*	10*
Carbon catabolism										
Calvin-Benson-Bassham cycle										
Embden-Meyerhof-parnas (EMP) pathway										
Pentose Phosphate pathway (PPP)										
Incomplete TCA cycle										
Nitrogen metabolism										
Nitrogen fixation										
Dissimilatory nitrate reduction										
Assimilatory nitrate reduction										
Sulfur metabolism										
Sulfur oxygenase reductase (SOR)	m			m						
Sulfur oxidation protein (Sox)	2	2	2	2	2	i	i			
Sulfite reductase										

m, perhaps missing; *i*, incomplete pathway. Besides, white box: absence of the gene/operon or pathway; gray box: presence of the gene/operon or pathway, the number of copies for each gene is indicated in white letterings.

1.4.2 Metabolic diversity of *Acidithiobacillus* spp

Effective acquisition of inorganic nutrients from the external environment is essential for autotrophic microbes to perform basic activities. *Acidithiobacillus* spp. are chemolithotrophic prokaryotes that inhabit acidic bioleaching environments and use energy and reducing power primarily from iron and/or sulfur oxidation to sustain essential pathways such as carbon and nitrogen assimilation.

1.4.2.1 Carbon metabolism

Carbon assimilation is the essential pathway of autotrophic bacteria inhabiting extreme environments. These microorganisms can use atmospheric carbon dioxide directly as their carbon source (Levicán et al., 2008).

Evidence showed that *Acidithiobacillus* spp., an obligate chemolithotroph, could incorporate CO₂ via the classical CBB cycle (also known as Calvin cycle) (Fig. 1.15). Compared with canonical forms of Rubisco, our results showed that *A. ferrooxidans* and *A. ferrivorans* had two copies of Rubisco form I gene (*cbbLS1* and *cbbLS2*) and one copy of form II gene (*cbbM*), whereas only one copy of form I as well as form II gene existed in *A. thiooxidans* and *A. caldus*. Previous studies indicated that *Hydrogenovibrio marinus*, the obligately lithoautotrophic hydrogen-oxidizing bacterium, could adapt well to environmental conditions with various levels of CO₂ concentrations owing to regulation mediated by Rubisco (Yoshizawa, Toyoda, Arai, Ishii, & Igarashi, 2004). Because Rubisco was the key enzyme in CO₂ fixation, *A. ferrooxidans* and *A. ferrivorans* might have an advantage in using atmospheric carbon dioxide compared with other two species.

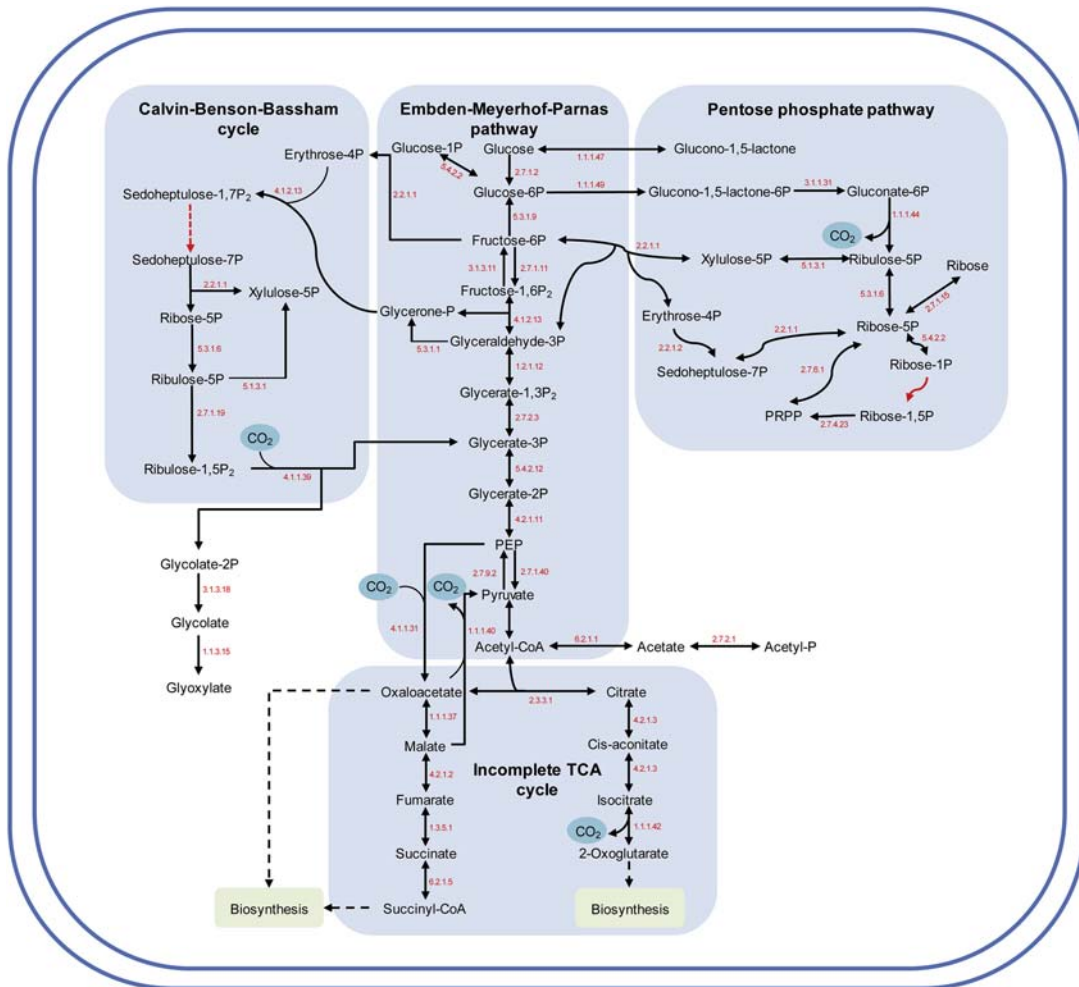


FIGURE 1.15 Predicted model of central carbon metabolism in all *Acidithiobacillus*, including Calvin-Benson-Bassham cycle, Embden-Meyerhof-Parnas pathway, pentose phosphate pathway, and incomplete tricarboxylic acid cycle (TCA) cycle. Details regarding the respective enzyme numbers and annotation classification are attached in Supplementary Material Table 1. Here, ribokinase (EC 2.7.1.15) was present only in *Acidithiobacillus thiooxidans*. Ribose 1,5-bisphosphokinase (EC 2.7.4.23) was absent in *Acidithiobacillus caldus* and *Acidithiobacillus ferrooxidans*. In addition, there is no malate dehydrogenase (EC 1.1.1.37) gene in *A. caldus*, *Acidithiobacillus thiooxidans* Licanantay, and *A. ferrooxidans* SS3. From Zhang X., She S., Dong W., Niu J., Xiao Y., Liang Y., Liu X., Zhang X., Fan F., Yin H. (2016). Comparative genomics unravels metabolic differences at the species and/or strain level and extremely acidic environmental adaptation of ten bacteria belonging to the genus *Acidithiobacillus*. *Systematic and Applied Microbiology*, 39(8), 493–502. <https://doi.org/10.1016/j.syapm.2016.08.007>.

Moreover, findings showed that the gene cluster of carboxysome including Rubisco form I genes was detected in *Acidithiobacillus* spp., and their corresponding proteins contained ribulose biphosphate carboxylase large chain, ribulose biphosphate carboxylase small chain, various copies of carboxysome shell protein (cso-carboxysomes), and carbon dioxide-concentrating protein CcmK (ccm-carboxysomes). However, the gene-encoding transcriptional initiation protein Tat was absent in *A. thiooxidans* and *A. caldus* (Fig. 1.16).

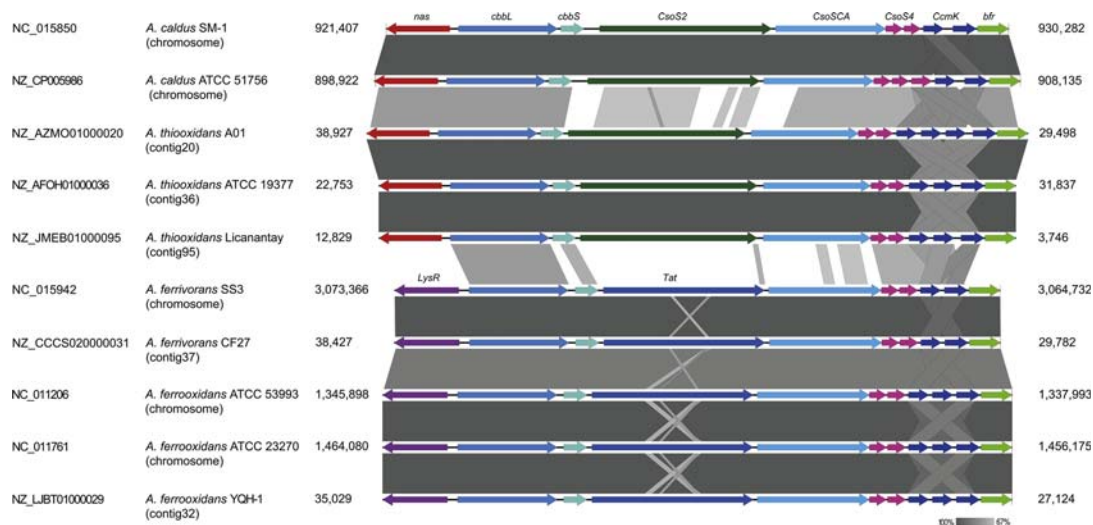


FIGURE 1.16 Visualization of carboxysome gene cluster in all 10 strains using the software Easyfig. *bfr*, bacterioferritin; *cbbL*, Rubisco, large chain; *cbbS*, Rubisco, small chain; *CcmK*, carbon dioxide-concentrating protein; *CsoS2*, carboxysome shell protein; *CsoS4*, carboxysome shell protein; *CsoSCA*, carboxysome shell protein; *LysR*, LysR family transcriptional regulator; *nas*, nitrate reductase; *Tat*, transcriptional initiation protein. From Zhang X., She S., Dong W., Niu J., Xiao Y., Liang Y., Liu X., Zhang X., Fan F., Yin H. (2016). Comparative genomics unravels metabolic differences at the species and/or strain level and extremely acidic environmental adaptation of ten bacteria belonging to the genus *Acidithiobacillus*. *Systematic and Applied Microbiology*, 39(8), 493–502. <https://doi.org/10.1016/j.syapm.2016.08.007>.

The carboxysome, a cytoplasmic polyhedral microcompartment, greatly increases carbon fixation in cyanobacteria and some chemoautotrophs (Klein et al., 2009). Here, carboxysome-associated carbonic anhydrase (CsoSCA) from *Halothiobacillus neapolitanus* (2fgy.1.A) was chosen for homology modeling (Fig. 1.17A). In all known instances, the zinc ligands of carbonic anhydrases (CAs) including α class, β class, and γ class CA, are composed of three histidine residues, or a combination of cysteine, histidine, and sometimes aspartate (So et al., 2004; Tripp, Smith & Ferry, 2001). In *A. thiooxidans* A01, homology modeling revealed that the catalytically essential zinc ion was probably coordinated to the CsoSCA apoprotein through His239, Cys250, and His253 (Fig. 1.17B). In addition, multiple sequence alignment indicated that carboxysome shell protein CsoSCA of *Halothiobacillus neapolitanus*, a novel carbonic anhydrase (ϵ -class CA) (So et al., 2004), shared amino acid sequence homology (range from 48% to 55%) with that of other strains belonging to genus *Acidithiobacillus* (Fig. 1.17C). Therefore, it was hypothesized that CsoSCA in *Acidithiobacillus* spp. might be a ϵ -class CA, which converted accumulated cytosolic bicarbonate into carbon dioxide, elevating the CO_2 concentration near the Rubisco (Price et al., 2004).

Genomic analyses indicated that a large number of genes related to the Embden–Meyerhof–Parnas (EMP) pathway and pentose phosphate pathway existed in the genome of *Acidithiobacillus* species (Fig. 1.15). The 3-phosphoglyceraldehyde generated from the CBB cycle enters the EMP pathway to produce glucose via anabolic reaction or provide carbon backbones for the generation of pyruvate via a catabolic reaction. As for the pentose phosphate pathway, ribokinase that catalyzes ribose 5-phosphate to ribose existed in

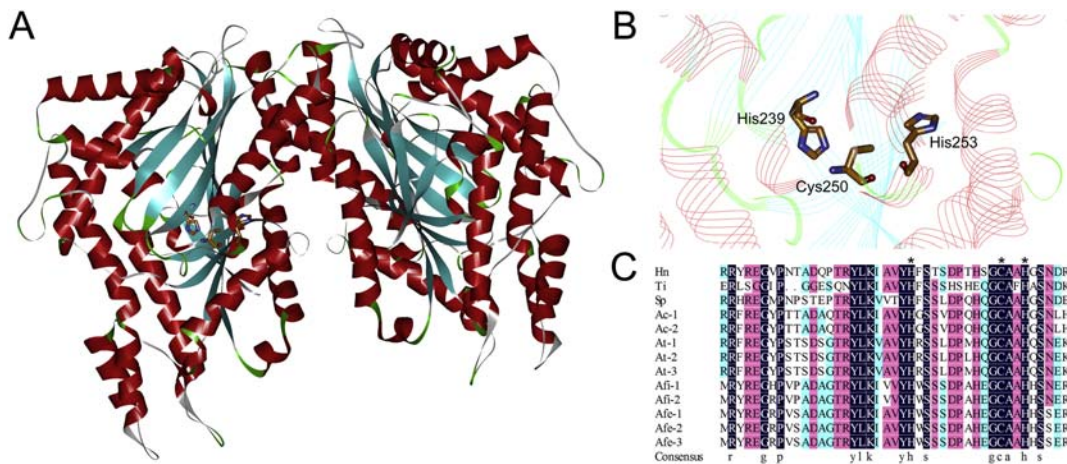


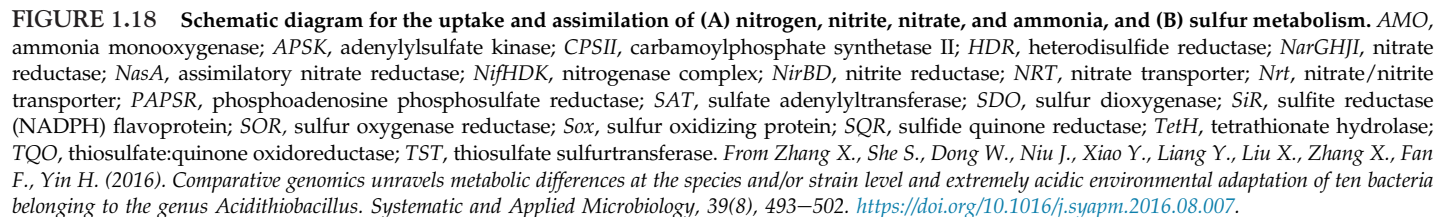
FIGURE 1.17 The ϵ -class carbonic anhydrase in *Acidithiobacillus*: (A) three-dimensional model of carbonic anhydrase (CA) in *Acidithiobacillus thiooxidans* A01; (B) the possible zinc ligand of CA; and (C) multiple sequence alignment of putative carboxysome-associated carbonic anhydrases from various bacteria. *Ac-1*, *Acidithiobacillus caldus* SM-1; *Ac-2*, *A. caldus* ATCC 51,756; *Afe-1*, *A. ferrooxidans* ATCC 53,993; *Afe-2*, *A. ferrooxidans* ATCC 23,270; *Afe-3*, *A. ferrooxidans* YQH-1; *Afi-1*, *A. ferrivorans* SS3; *Afi-2*, *A. ferrivorans* CF27; *At-1*, *A. thiooxidans* A01; *At-2*, *A. thiooxidans* ATCC 19,377; *At-3*, *A. thiooxidans* Licanantay; *Hn*, *Halothiobacillus neapolitanus* c2; *Sp*, *Synechococcus* sp. WH 8102; *Ti*, *Thiomonas intermedia* K12. Black asterisk indicates potential zinc-binding residues. From Zhang X., She S., Dong W., Niu J., Xiao Y., Liang Y., Liu X., Zhang X., Fan F., Yin H. (2016). Comparative genomics unravels metabolic differences at the species and/or strain level and extremely acidic environmental adaptation of ten bacteria belonging to the genus *Acidithiobacillus*. *Systematic and Applied Microbiology*, 39(8), 493–502. <https://doi.org/10.1016/j.syapm.2016.08.007>.

only *A. thiooxidans*. In addition, ribose 1,5-bisphosphokinase that catalyzes ribose 1,5-bisphosphate to 5-phosphoribosyl diphosphate was absent in *A. caldus* and *A. ferrivorans*. As stated by (Valdés et al., 2008b), an incomplete TCA cycle that lacks 2-oxoglutarate dehydrogenase complex was documented in all obligate chemoautotrophs. In this study, our results showed the absence of gene-encoding malate dehydrogenase in *A. caldus* as well as *A. thiooxidans* Licanantay and *A. ferrivorans* SS3. Through an incomplete TCA cycle, pyruvate would be converted to succinyl-CoA and 2-oxoglutarate as metabolites associated with the biosynthesis of cellular components (Wood et al., 2004).

1.4.2.2 Nitrogen metabolism

The uptake of nitrogen, such as nitrogen fixation, and assimilation of nitrate, nitrite, and ammonia have a key role in the ecophysiology of microbiological communities. Therefore, it is necessary to understand the nitrogen metabolism of these microbes in extremely acidic environments.

Similar to other diazotrophic bacteria, *A. ferrivorans* and *A. ferrooxidans* depend on a nitrogenase complex that can fix atmospheric nitrogen under anaerobic or microaerobic conditions (Valdés et al., 2008a). In *A. thiooxidans* and *A. caldus*, however, there are no genes for N_2 fixation (*nifHDK*). Instead, our results showed the presence of genes encoding nitrate reductase and nitrite reductase. Thus, alternative mechanisms that might use environmental nitrate and nitrite as the nitrogen source existed in these two species, satisfying their nitrogen demands (Fig. 1.18A).



In *A. caldus* SM-1 but not *A. caldus* ATCC 51,756, the genomic region associated with nitrate reduction, which encodes nitrate reductase subunit alpha, NADH large subunit, NADH small subunit, major facilitator superfamily (MFS) transporter, and nitrate transporter, was identified (Fig. 1.19A). Similar to *A. caldus* genome, the 2 *A. ferrivorans* strains and *A. thiooxidans*, except for *A. thiooxidans* ATCC 19,377, contained the gene cluster involved

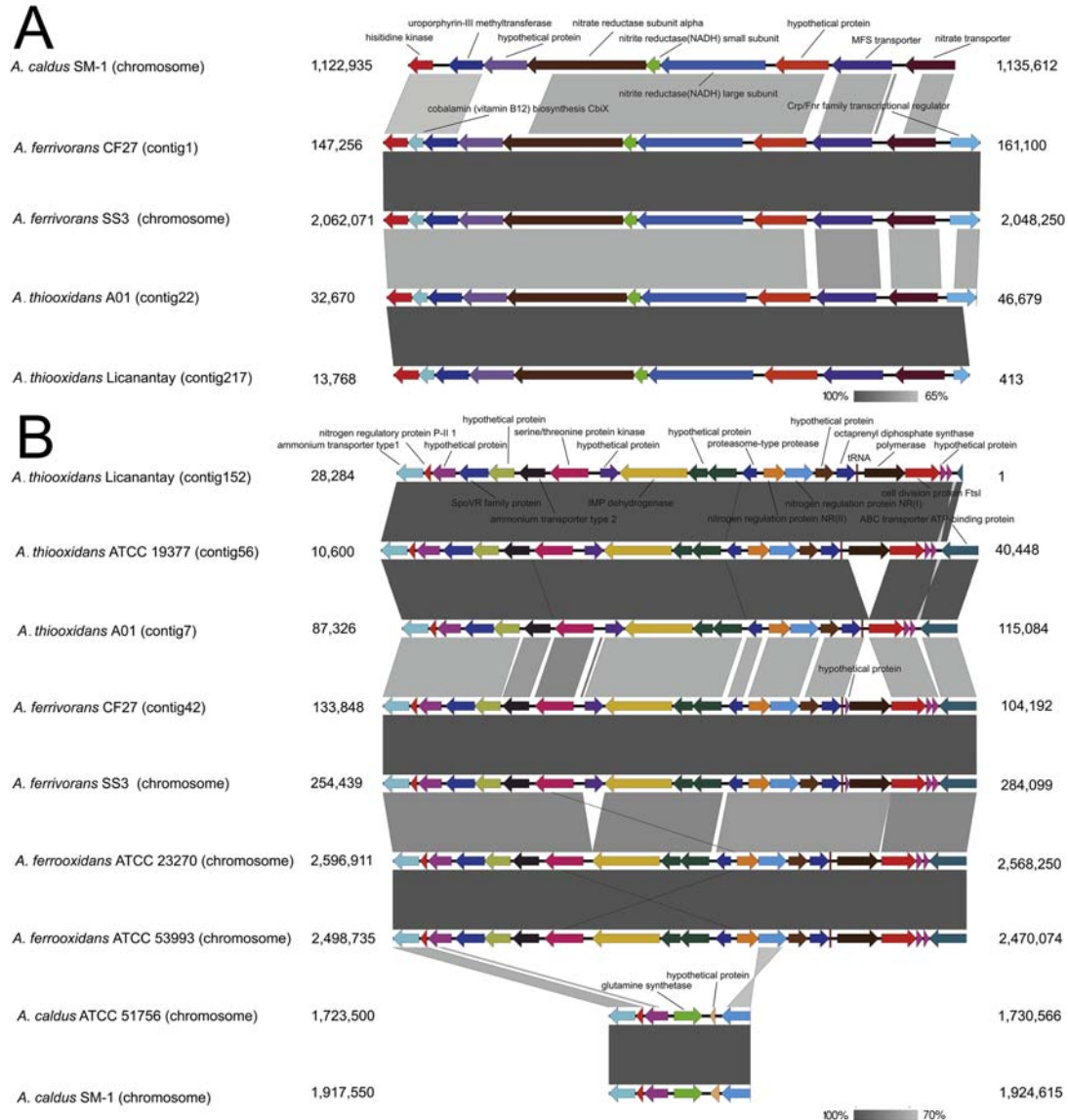


FIGURE 1.19 Predicted operons involved in putative (A) nitrate assimilation genes as well as (B) ammonium transporters. From Zhang X., She S., Dong W., Niu J., Xiao Y., Liang Y., Liu X., Zhang X., Fan F., Yin H. (2016). Comparative genomics unravels metabolic differences at the species and/or strain level and extremely acidic environmental adaptation of ten bacteria belonging to the genus *Acidithiobacillus*. *Systematic and Applied Microbiology*, 39(8), 493–502. <https://doi.org/10.1016/j.syapm.2016.08.007>.

in nitrate reduction. Also, the *narGHJ* operon (which encoded nitrate reductase A subunit α , subunit β , subunit δ , and subunit γ , respectively) was also found in all species except for *A. ferrooxidans*. With respect to assimilatory nitrate reduction, *A. thiooxidans*, *A. caldus*, and *A. ferrivorans* had the gene *nasA* encoding the assimilatory nitrate reductase catalytic subunit. However, no gene *nasB*, which encodes the electron transfer subunit in *Bacillus subtilis* (Ogawa et al., 1995), was identified in these species, which makes the electron donor unclear.

Several genes encoding nitrate/nitrite transporter and nitrate transporter, which transfer extracellular nitrate or nitrite into the cell, were also found in *A. thiooxidans*, *A. caldus*, and *A. ferrivorans*. In addition, results showed that all *Acidithiobacillus* species would incorporate ammonia by the ammonia transporter, which has been reported in almost all organisms including bacteria (Merrick et al., 2006). The genes *amt1* and *amt2* in *A. ferrooxidans*, *A. thiooxidans*, and *A. ferrivorans* were located in a gene cluster *amt1-glnK1-hyp-spoVR-hyp-amt2*, whereas the gene cluster *amt1-glnK1-hyp-glnA-hyp-ntrC*, which lacked gene *amt2*, was detected in *A. caldus* (Fig. 1.19B). Moreover, gene *amtB* encoding ammonia permease that participates in ammonia uptake under low nitrogen levels (Levicán et al., 2008) was also identified.

Our results revealed that nitrilase existed in all *Acidithiobacillus* species, whereas the gene encoding putative ammonia monooxygenase was found only in *A. thiooxidans*. Moreover, although the gene encoding carbamoylphosphate synthetase I that catalyzes ammonia to carbamoyl phosphate (carbamoyl-P) was absent in all *Acidithiobacillus*, the carbamoylphosphate synthetase II (CPSII) that performs the catalysis of L-glutamine to generate carbamoyl-P was identified. Through a series of enzyme reactions catalyzed by glutamine synthetase type I, glutamate synthase (large subunit GltB and small subunit GltD), and CPSII (small subunit CarA and large subunit CarB), ammonia would enter into the biosynthesis of amino acids and other vital compounds (Fig. 1.18A).

1.4.2.3 Sulfur metabolism

In recent decades, models of sulfur oxidation in *Acidithiobacillus* genus have been well-studied (Chen et al., 2012; Mangold, Valdés, Holmes, & Dopson, 2011; Valdés et al., 2008a; Yin et al., 2014b). Regarding sulfite oxidation in the cytoplasm, one possibility is that sulfite was initially catalyzed by the well-characterized APS reductase complex (Hipp et al., 1997; Meyer & Kuever, 2007, 2008) to produce APS. However, all *Acidithiobacillus* spp. lacked genes *aprBA* encoding APS reductase and sulfite oxidase that directly oxidizes sulfite to sulfate (Rohwerder et al., 2003). Thus, the putative sulfite oxidation in these species was: sulfite was catalyzed to APS via an unknown mechanism, and then to sulfate (Fig. 1.18B). In particular, our studies showed that the fused SAT/adenylylsulfate kinase (APSK) (bifunctional enzyme with sulfate adenylyltransferase and adenylylsulfate kinase activities) in all *Acidithiobacillus* had a conserved ATP-sulfurylase domain (pfam01747), part of which was a bifunctional polypeptide chain in association with APSK (pfam01583) (Kurima et al., 1998; Rosenthal & Leustek, 1995). Interestingly, the gene *sor* could not be identified in *A. thiooxidans* ATCC 19,377 and *A. caldus* SM-1. It seems that the absence of gene *sor* in *A. thiooxidans* ATCC 19,377 is reasonable, mainly because our annotation results are based on the draft genome of this strain. As for *sor* in *A. caldus*, it remains to be further validated. In addition, an incomplete Sox system (*soxY*, *soxZ*, and *soxB*) was found in *A. ferrivorans*, probably indicating functional loss in this species.

As for assimilatory sulfate reduction, gene-encoding phosphoadenosine phosphosulfate reductase was absent in *A. caldus* and *A. thiooxidans* ATCC 19,377. In addition, sulfite reductase that catalyzes sulfite to sulfide was found in *A. ferrooxidans* and *A. ferrivorans*. Interestingly, genes encoding sulfite reductase were identified in *A. thiooxidans* Licanantay, suggesting that horizontal gene transfer event might occur in this strain. Considering the absence of sulfite reductase in *A. caldus* and *A. thiooxidans* except for *A. thiooxidans* Licanantay, the model proposed in our study was that SOR would be used as a replacement for sulfite reductase to compensate for the cytoplasmic sulfide, which was involved in cysteine biosynthesis (Fig. 1.18B).

1.4.3 Conclusions

Comparative genomic analysis reveals the common and distinctive features of 10 bio-leaching bacterial strains from similar habitats and provides several references to improve our understanding of the potential mechanisms that enable acidophiles to adapt to extremely acidic environments. Based on the genomic contents of *Acidithiobacillus* species, it was shown that different metabolic pathways might allow these microorganisms to acquire energy, a carbon source, and a nitrogen source from acidic environments, ensuring their survival and proliferation in these harsh environments.

Acknowledgments

We thank the National Center for Biotechnology Information (NCBI) for providing the genome sequences. In addition, we gratefully acknowledge financial support from the National Natural Science Foundation of China (31570113 and 41573072) and the Natural Science Foundation of Hunan Province (2020JJ5709).

References

- Acuña, L. G., Cárdenas, J. P., Covarrubias, P. C., Haristoy, J. J., Flores, R., Nuñez, H., et al. (2013). Architecture and gene repertoire of the flexible genome of the extreme acidophile *Acidithiobacillus caldus*. *PLoS One*, 8(11). <https://doi.org/10.1371/journal.pone.0078237>.
- Adriaenssens, E. M., Van Zyl, L., De Maayer, P., Rubagotti, E., Rybicki, E., Tuffin, M., et al. (2015). Metagenomic analysis of the viral community in Namib Desert hypoliths. *Environmental Microbiology*, 17(2), 480–495. <https://doi.org/10.1111/1462-2920.12528>.
- Aoshima, M., Ishii, M., & Igarashi, Y. (2004). A novel enzyme, citryl-CoA synthetase, catalysing the first step of the citrate cleavage reaction in *Hydrogenobacter thermophilus* TK-6. *Molecular Microbiology*, 52(3), 751–761. <https://doi.org/10.1111/j.1365-2958.2004.04009.x>.
- Arrigo, K. R. (2005). Marine microorganisms and global nutrient cycles. *Nature*, 437(7057), 349–355. <https://doi.org/10.1038/nature04159>.
- Baker-Austin, C., & Dopson, M. (2007). Life in acid: pH homeostasis in acidophiles. *Trends in Microbiology*, 15(4), 165–171. <https://doi.org/10.1016/j.tim.2007.02.005>.
- Baker, B. J., & Banfield, J. F. (2003). Microbial communities in acid mine drainage. *FEMS Microbiology Ecology*, 44(2), 139–152. [https://doi.org/10.1016/S0168-6496\(03\)00028-X](https://doi.org/10.1016/S0168-6496(03)00028-X).
- Bentley, S. D., & Parkhill, J. (2004). Comparative genomic structure of prokaryotes. *Annual Review of Genetics*, 38, 771–792. <https://doi.org/10.1146/annurev.genet.38.072902.094318>.
- Berg, I. A. (2011). Ecological aspects of the distribution of different autotrophic CO₂ fixation pathways. *Applied and Environmental Microbiology*, 77(6), 1925–1936. <https://doi.org/10.1128/AEM.02473-10>.
- Berg, I. A., Kockelkorn, D., Ramos-Vera, W. H., Say, R. F., Zarzycki, J., Hügler, M., et al. (2010). Autotrophic carbon fixation in archaea. *Nature Reviews Microbiology*, 8(6), 447–460. <https://doi.org/10.1038/nrmicro2365>.

- Bertin, P. N., Heinrich-Salmeron, A., Pelletier, E., Goulhen-Chollet, F., Arsène-Ploetze, F., Gallien, S., et al. (2011). Metabolic diversity among main microorganisms inside an arsenic-rich ecosystem revealed by meta- and proteo-genomics. *The ISME Journal*, 5(11), 1735–1747. <https://doi.org/10.1038/ismej.2011.51>.
- Bird, L. J., Bonnefoy, V., & Newman, D. K. (2011). Bioenergetic challenges of microbial iron metabolisms. *Trends in Microbiology*, 19(7), 330–340. <https://doi.org/10.1016/j.tim.2011.05.001>.
- Bogdanova, T. I., Tsaplina, I. A., Kondrat'eva, T. F., Duda, V. I., Suzina, N. E., Melamud, V. S., et al. (2006). *Sulfobacillus thermotolerans* sp. nov., a thermotolerant, chemolithotrophic bacterium. *International Journal of Systematic and Evolutionary Microbiology*, 56(5), 1039–1042. <https://doi.org/10.1099/ijs.0.64106-0>.
- Bonnefoy, V., & Holmes, D. S. (2012). Genomic insights into microbial iron oxidation and iron uptake strategies in extremely acidic environments. *Environmental Microbiology*, 14(7), 1597–1611. <https://doi.org/10.1111/j.1462-2920.2011.02626.x>.
- Bosecker, K., Mengel-Jung, G., & Schippers, A. (2004). In M. Tsezos, A. Hatzikioseyan, & E. Remoundaki (Eds.), *Biohydrometallurgy: A sustainable technology in Evolution* (pp. 585–593). University of Athens. <https://doi.org/10.1111/1462-2920.12114>.
- Breitbart, M., Salamon, P., Andresen, B., Mahaffy, J. M., Segall, A. M., Mead, D., et al. (2002). Genomic analysis of uncultured marine viral communities. *Proceedings of the National Academy of Sciences of the United States of America*, 99(22), 14250–14255. <https://doi.org/10.1073/pnas.202488399>.
- Breuker, A., Blazejak, A., Bosecker, K., & Schippers, A. (2009). Diversity of iron oxidizing bacteria from various sulfidic mine waste dumps. *Advanced Materials Research*, 71–73, 47–50. <https://doi.org/10.4028/www.scientific-net/AMR.71-73.47>.
- Brierley, C. L. (2001). Bacterial succession in bioheap leaching. *Hydrometallurgy*, 59(2–3), 249–255. [https://doi.org/10.1016/S0304-386X\(00\)00171-7](https://doi.org/10.1016/S0304-386X(00)00171-7).
- Cárdenas, J. P., Ortiz, R., Norris, P. R., Watkin, E., & Holmes, D. S. (2015). Reclassification of '*Thiobacillus prosperus*' Huber and Stetter 1989 as *Acidihalobacter prosperus* gen. nov., sp. nov., a member of the family Ectothiorhodospiraceae. *International Journal of Systematic and Evolutionary Microbiology*, 65(10), 3641–3644. <https://doi.org/10.1099/ijs.0.000468>.
- Cárdenas, J. P., Quatrini, R., & Holmes, D. S. (2016). Genomic and metagenomic challenges and opportunities for bioleaching: A mini-review. *Research in Microbiology*, 167(7), 529–538. <https://doi.org/10.1016/j.resmic.2016.06.007>.
- Castelle, C. J., Roger, M., Bauzan, M., Brugna, M., Lignon, S., Nimtz, M., et al. (2015). The aerobic respiratory chain of the acidophilic archaeon *Ferroplasma acidiphilum*: A membrane-bound complex oxidizing ferrous iron. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 1847(8), 717–728. <https://doi.org/10.1016/j.bbabi.2015.04.006>.
- Chen, L. X., Huang, L. N., Méndez-García, C., Kuang, J. L., Hua, Z. S., Liu, J., et al. (2016). Microbial communities, processes and functions in acid mine drainage ecosystems. *Current Opinion in Biotechnology*, 38, 150–158. <https://doi.org/10.1016/j.copbio.2016.01.013>.
- Chen, L. X., Hu, M., Huang, L. N., Hua, Z. S., Kuang, J. L., Li, S. J., et al. (2015). Comparative metagenomic and meta-transcriptomic analyses of microbial communities in acid mine drainage. *The ISME Journal*, 9(7), 1579–1592. <https://doi.org/10.1038/ismej.2014.245>.
- Chen, L. X., Li, J. T., Chen, Y. T., Huang, L. N., Hua, Z. S., Hu, M., et al. (2013). Shifts in microbial community composition and function in the acidification of a lead/zinc mine tailings. *Environmental Microbiology*, 15(9), 2431–2444. <https://doi.org/10.1111/1462-2920.12114>.
- Chen, L., Ren, Y., Lin, J., Liu, X., Pang, X., & Lin, J. (2012). *Acidithiobacillus caldus* sulfur oxidation model based on transcriptome analysis between the wild type and sulfur oxygenase reductase defective mutant. *PLoS One*, 7(9). <https://doi.org/10.1371/journal.pone.0039470>.
- Coram, N. J., & Rawlings, D. E. (2002). Molecular relationship between two groups of the genus *Leptospirillum* and the finding that *Leptospirillum ferroplum* sp. nov. Dominates south African commercial biooxidation tanks that operate at 40°C. *Applied and Environmental Microbiology*, 68(2), 838–845. <https://doi.org/10.1128/AEM.68.2.838-845.2002>.
- Craig, J. W., Cherry, M. A., & Brady, S. F. (2011). Long-chain N-acyl amino acid synthases are linked to the putative PEP-CTERM/exosortase protein-sorting system in gram-negative bacteria. *Journal of Bacteriology*, 193(20), 5707–5715. <https://doi.org/10.1128/JB.05426-11>.
- Demergasso, C. S., Galleguillos, P., Pedro, A., Escudero, G., Lorena, V., Zepeda, A., et al. (2005). Molecular characterization of microbial populations in a low-grade copper ore bioleaching test heap. *Hydrometallurgy*, 80(4), 241–253. <https://doi.org/10.1016/j.hydromet.2005.07.013>.

- Denef, V. J., Mueller, R. S., & Banfield, J. F. (2010). AMD biofilms: Using model communities to study microbial evolution and ecological complexity in nature. *The ISME Journal*, 4(5), 599–610. <https://doi.org/10.1038/ismej.2009.158>.
- Dick, G. J., Andersson, A. F., Baker, B. J., Simmons, S. L., Thomas, B. C., Yelton, A. P., et al. (2009). Community-wide analysis of microbial genome sequence signatures. *Genome Biology*, 10(8). <https://doi.org/10.1186/gb-2009-10-8-r85>.
- Dopson, M., & Johnson, D. B. (2012). Biodiversity, metabolism and applications of acidophilic sulfur-metabolizing microorganisms. *Environmental Microbiology*, 14(10), 2620–2631. <https://doi.org/10.1111/j.1462-2920.2012.02749.x>.
- Emerson, D., Fleming, E. J., & McBeth, J. M. (2010). Iron-oxidizing bacteria: An environmental and genomic perspective. *Annual Review of Microbiology*, 64, 561–583. <https://doi.org/10.1146/annurev.micro.112408.134208>.
- Falagán, C., Sánchez-España, J., & Johnson, D. B. (2014). New insights into the biogeochemistry of extremely acidic environments revealed by a combined cultivation-based and culture-independent study of two stratified pit lakes. *FEMS Microbiology Ecology*, 87(1), 231–243. <https://doi.org/10.1111/1574-6941.12218>.
- Fancello, L., Trape, S., Robert, C., Boyer, M., Popgeorgiev, N., Raoult, D., et al. (2013). Viruses in the desert: A metagenomic survey of viral communities in four perennial ponds of the Mauritanian Sahara. *The ISME Journal*, 7(2), 359–369. <https://doi.org/10.1038/ismej.2012.101>.
- Forstner, K. U., von Mering, C., Hooper, S. D., & Bork, P. (2005). Environments shape the nucleotide composition of genomes. *EMBO Reports*, 6(12), 1208–1213. <https://doi.org/10.1038/sj.embor.7400538>.
- Franceschini, A., Szklarczyk, Frankild, S., Kuhn, M., Simonovic, M., Roth, A., Lin, J., et al. (2013). STRING v9. 1: Protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Research*, 41(D1).
- Franke, & Rensing. (2007). Acidophiles: Mechanisms to tolerate metal and acid toxicity. In *Physiology and biochemistry of extremophiles* (pp. 271–277).
- Friedrich, M. W. (2005). Stable-isotope probing of DNA: Insights into the function of uncultivated microorganisms from isotopically labeled metagenomes. *Current Opinion in Biotechnology*, 17(1), 59–66. <https://doi.org/10.1016/j.copbio.2005.12.003>.
- Fujimura, R., Sato, Y., Nishizawa, T., Oshima, K., Kim, S. W., Hattori, M., et al. (2012). Complete genome sequence of *Leptospirillum ferrooxidans* strain C2-3, isolated from a fresh volcanic ash deposit on the Island of Miyake, Japan. *Journal of Bacteriology*, 194(15), 4122–4123. <https://doi.org/10.1128/JB.00696-12>.
- Goltsman, D. S. A., Comolli, L. R., Thomas, B. C., & Banfield, J. F. (2015). Community transcriptomics reveals unexpected high microbial diversity in acidophilic biofilm communities. *The ISME Journal*, 9, 1014–1023. <https://doi.org/10.1038/ismej.2014.200>.
- Goltsman, D. S. A., Dasari, M., Thomas, B. C., Shah, M. B., VerBerkmoes, N. C., & Hettich, R. L. (2013). New group in the *Leptospirillum* clade: Cultivation-independent community genomics, proteomics, and transcriptomics of the new species “*Leptospirillum* group IV UBA BS. *Applied and Environmental Microbiology*, 79, 5384–5393. <https://doi.org/10.1128/aem.00202-13>.
- González-Toril, E., Llobet-Brossa, E., Casamayor, E. O., Amann, R., & Amils, R. (2003). Microbial ecology of an extreme acidic environment, the Tinto River. *Applied and Environmental Microbiology*, 69(8), 4853–4865. <https://doi.org/10.1128/AEM.69.8.4853-4865.2003>.
- González, A., Bellenberg, S., Mamani, S., Ruiz, L., Echeverría, A., Soullère, L., et al. (2013). AHL signaling molecules with a large acyl chain enhance biofilm formation on sulfur and metal sulfides by the bioleaching bacterium *Acidithiobacillus ferrooxidans*. *Applied Microbiology and Biotechnology*, 97(8), 3729–3737. <https://doi.org/10.1007/s00253-012-4229-3>.
- Guazzaroni, M. E., Morgante, V., Mirete, S., & González-Pastor, J. E. (2013). Novel acid resistance genes from the metagenome of the Tinto River, an extremely acidic environment. *Environmental Microbiology*, 15(4), 1088–1102. <https://doi.org/10.1111/1462-2920.12021>.
- Guo, X., Yin, H., Cong, J., Dai, Z., Dai, Y., & Liu, X. (2013). RubisCO gene clusters found in a metagenome microarray from acid mine drainage. *Applied and Environmental Microbiology*, 79(6), 2019–2026. <https://doi.org/10.1128/AEM.03400-12>.
- Guo, X., Yin, H., Liang, Y., Hu, Q., Zhou, X., Xiao, Y., et al. (2014). Comparative genome analysis reveals metabolic versatility and environmental adaptations of *Sulfobacillus thermosulfidooxidans* strain ST. *PloS One*, 9(6). <https://doi.org/10.1371/journal.pone.0099417>.
- Gupta, R., & Schuster, M. (2013). Negative regulation of bacterial quorum sensing tunes public goods cooperation. *The ISME Journal*, 7(11), 2159–2168. <https://doi.org/10.1038/ismej.2013.109>.

- Haft, D. H., Paulsen, I. T., Ward, N., & Selengut, J. D. (2006). Exopolysaccharide-associated protein sorting in environmental organisms: The PEP-CTERM/SpsH system. Application of a novel phylogenetic profiling heuristic. *BMC Biology*, 4. <https://doi.org/10.1186/1741-7007-4-29>.
- Hallberg, K. B. (2010). New perspectives in acid mine drainage microbiology. *Hydrometallurgy*, 104(3–4), 448–453. <https://doi.org/10.1016/j.hydromet.2009.12.013>.
- Hallberg, K. B., González-Toril, E., & Johnson, D. B. (2010). *Acidithiobacillus ferrivorans*, sp. nov.; facultatively anaerobic, psychrotolerant iron- and sulfur-oxidizing acidophiles isolated from metal mine-impacted environments. *Extremophiles*, 14(1), 9–19. <https://doi.org/10.1007/s00792-009-0282-y>.
- Hallberg, K. B., & Johnson, D. B. (2001). Biodiversity of acidophilic prokaryotes. *Advances in Applied Microbiology*, 49, 37–84. [https://doi.org/10.1016/S0065-2164\(01\)49009-5](https://doi.org/10.1016/S0065-2164(01)49009-5).
- Handelsman, J. (2004). Metagenomics: Application of genomics to uncultured microorganisms. *Microbiology and Molecular Biology Reviews*, 68(4), 669–685. <https://doi.org/10.1128/MMBR.68.4.669-685.2004>.
- Harris, J. (2009). Soil microbial communities and restoration ecology: Facilitators or followers? *Science*, 325(5940), 573–574. <https://doi.org/10.1126/science.1172975>.
- Hedrich, S., Schlömann, M., & Barrie Johnson, D. (2011). The iron-oxidizing proteobacteria. *Microbiology*, 157(6), 1551–1564. <https://doi.org/10.1099/mic.0.045344-0>.
- He, Z., Xu, M., Deng, Y., Kang, S., Kellogg, L., Wu, L., et al. (2010). Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO₂. *Ecology Letters*, 13(5), 564–575. <https://doi.org/10.1111/j.1461-0248.2010.01453.x>.
- Hildebrand, F., Meyer, A., & Eyre-Walker, A. (2010). Evidence of selection upon genomic GC-content in bacteria. *PLoS Genetics*, 6(9). <https://doi.org/10.1371/journal.pgen.1001107>.
- Hipp, W. M., Pott, A. S., Thum-Schmitz, N., Faath, I., Dahl, C., & Trüper, H. G. (1997). Towards the phylogeny of APS reductases and sirohaem sulfite reductases in sulfate-reducing and sulfur-oxidizing prokaryotes. *Microbiology*, 143(9), 2891–2902. <https://doi.org/10.1099/00221287-143-9-2891>.
- Hua, Z. S., Han, Y. J., Chen, L. X., Liu, J., Hu, M., Li, S. J., et al. (2015). Ecological roles of dominant and rare prokaryotes in acid mine drainage revealed by metagenomics and metatranscriptomics. *The ISME Journal*, 9(6), 1280–1294. <https://doi.org/10.1038/ismej.2014.212>.
- Huang, L. N., Zhou, W. H., Hallberg, K. B., Wan, C. Y., Li, J., & Shu, W. S. (2011). Spatial and temporal analysis of the microbial community in the tailings of a Pb-Zn mine generating acidic drainage. *Applied and Environmental Microbiology*, 77(15), 5540–5544. <https://doi.org/10.1128/AEM.02458-10>.
- Hügler, M., Huber, H., Molyneaux, S. J., Vetriani, C., & Sievert, S. M. (2007). Autotrophic CO₂ fixation via the reductive tricarboxylic acid cycle in different lineages within the phylum Aquificae: Evidence for two ways of citrate cleavage. *Environmental Microbiology*, 9(1), 81–92. <https://doi.org/10.1111/j.1462-2920.2006.01118.x>.
- Hügler, M., Wirsén, C. O., Fuchs, G., Taylor, C. D., & Sievert, S. M. (2005). Evidence for autotrophic CO₂ fixation via the reductive tricarboxylic acid cycle by members of the ϵ subdivision of proteobacteria. *Journal of Bacteriology*, 187(9), 3020–3027. <https://doi.org/10.1128/JB.187.9.3020-3027.2005>.
- Ilbert, M., & Bonnefoy, V. (2013). Insight into the evolution of the iron oxidation pathways. *Biochimica et Biophysica Acta - Bioenergetics*, 1827(2), 161–175. <https://doi.org/10.1016/j.bbabi.2012.10.001>.
- Jacobsen, A., Hendriksen, R. S., Aaresturp, F. M., Ussery, D. W., & Friis, C. (2011). The *Salmonella enterica* pan-genome. *Microbial Ecology*, 62(3), 487–504. <https://doi.org/10.1007/s00248-011-9880-1>.
- Jiang, H., Liang, Y., Yin, H., Xiao, Y., Guo, X., & Xu, Y. (2015). Effects of arsenite resistance on the growth and functional gene expression of *Leptospirillum ferriphilum* and *Acidithiobacillus thiooxidans* in pure culture and coculture. *BioMed Research International*, 1–13. <https://doi.org/10.1155/2015/203197>.
- Jiao, N., Herndl, G. J., Hansell, D. A., Benner, R., Kattner, G., Wilhelm, S. W., et al. (2010). Microbial production of recalcitrant dissolved organic matter: Long-term carbon storage in the global ocean. *Nature Reviews Microbiology*, 8(8), 593–599. <https://doi.org/10.1038/nrmicro2386>.
- Johnson, D. B. (1998). Biodiversity and ecology of acidophilic microorganisms. *FEMS Microbiology Ecology*, 27(4), 307–317. [https://doi.org/10.1016/S0168-6496\(98\)00079-8](https://doi.org/10.1016/S0168-6496(98)00079-8).
- Johnson, S. S., Chevrette, M. G., Ehlmann, B. L., & Benison, K. C. (2015). Insights from the metagenome of an acid salt lake: The role of biology in an extreme depositional environment. *PLoS One*, 10(4). <https://doi.org/10.1371/journal.pone.0122869>.
- Johnson, D. B., & Hallberg, K. B. (2003). The microbiology of acidic mine waters. *Research in Microbiology*, 154(7), 466–473. [https://doi.org/10.1016/S0923-2508\(03\)00114-1](https://doi.org/10.1016/S0923-2508(03)00114-1).

- Johnson, D., & Hallberg, K. (2008). Carbon, iron and sulfur metabolism in acidophilic micro-organisms. *Advances in Microbial Physiology*, 54, 201–255. [https://doi.org/10.1016/S0065-2911\(08\)00003-9](https://doi.org/10.1016/S0065-2911(08)00003-9).
- Johnson, D. B., Joulain, C., D'Hugues, P., & Hallberg, K. B. (2008). *Sulfobacillus benefaciens* sp. nov., an acidophilic facultative anaerobic Firmicute isolated from mineral bioleaching operations. *Extremophiles*, 12(6), 789–798. <https://doi.org/10.1007/s00792-008-0184-4>.
- Jones, C. M., Stres, B., Rosenquist, M., & Hallin, S. (2008). Phylogenetic analysis of nitrite, nitric oxide, and nitrous oxide respiratory enzymes reveal a complex evolutionary history for denitrification. *Molecular Biology and Evolution*, 25(9), 1955–1966. <https://doi.org/10.1093/molbev/msn146>.
- Justice, N. B., Norman, A., Brown, C. T., Singh, A., Thomas, B. C., & Banfield, J. F. (2014). Comparison of environmental and isolate *Sulfobacillus* genomes reveals diverse carbon, sulfur, nitrogen, and hydrogen metabolisms. *BMC Genomics*, 15(1). <https://doi.org/10.1186/1471-2164-15-1107>.
- Klein, M. G., Zwart, P., Bagby, S. C., Cai, F., Chisholm, S. W., Heinhorst, S., et al. (2009). Identification and structural analysis of a novel carboxysome shell protein with implications for metabolite transport. *Journal of Molecular Biology*, 392(2), 319–333. <https://doi.org/10.1016/j.jmb.2009.03.056>.
- Kock, D., & Schippers, A. (2006). Geomicrobiological investigation of two different mine waste tailings generating acid mine drainage. *Hydrometallurgy*, 83(1–4), 167–175. <https://doi.org/10.1016/j.hydromet.2006.03.022>.
- Kock, D., & Schippers, A. (2008). Quantitative microbial community analysis of three different sulfidic mine tailing dumps generating acid mine drainage. *Applied and Environmental Microbiology*, 74(16), 5211–5219. <https://doi.org/10.1128/AEM.00649-08>.
- Kubicek, C. P., Herrera-Estrella, A., Seidl-Seiboth, V., Martinez, D. A., Druzhinina, I. S., Thon, M., et al. (2011). Comparative genome sequence analysis underscores mycoparasitism as the ancestral life style of *Trichoderma*. *Genome Biology*, 12(4). <https://doi.org/10.1186/gb-2011-12-4-r40>.
- Kurima, K., Warman, M. L., Krishnan, S., Domowicz, M., Krueger, R. C., Deyrup, A., et al. (1998). A member of a family of sulfate-activating enzymes causes murine brachymorphism. *Proceedings of the National Academy of Sciences of the United States of America*, 8681–8685. <https://doi.org/10.1073/pnas.95.15.8681>.
- Levicán, G., Ugalde, J. A., Ehrenfeld, N., Maass, A., & Parada, P. (2008). Comparative genomic analysis of carbon and nitrogen assimilation mechanisms in three indigenous bioleaching bacteria: Predictions and validations. *BMC Genomics*, 9. <https://doi.org/10.1186/1471-2164-9-581>.
- Liang, J., Bai, Y., Men, Y., & Qu, J. (2017). Microbe-microbe interactions trigger Mn(II)-oxidizing gene expression. *The ISME Journal*, 11(1), 67–77. <https://doi.org/10.1038/ismej.2016.106>.
- Li, M., Chen, Z., Zhang, P., Pan, X., Jiang, C., An, X., et al. (2008). Crystal structure studies on sulfur oxygenase reductase from *Acidianus tengchongensis*. *Biochemical and Biophysical Research Communications*, 369(3), 919–923. <https://doi.org/10.1016/j.bbrc.2008.02.131>.
- Li, J., & Gu, J. D. (2007). Complete degradation of dimethyl isophthalate requires the biochemical cooperation between *Klebsiella oxytoca* Sc and *Methylobacterium mesophilicum* Sr Isolated from Wetland sediment. *The Science of the Total Environment*, 380(1–3), 181–187. <https://doi.org/10.1016/j.scitotenv.2006.12.033>.
- Liljeqvist, M., Ossandon, F. J., González, C., Rajan, S., Stell, A., Valdes, J., et al. (2015). Metagenomic analysis reveals adaptations to a cold-adapted lifestyle in a low-temperature acid mine drainage stream. *FEMS Microbiology Ecology*, 91(4). <https://doi.org/10.1093/femsec/fiv011>.
- Liljeqvist, M., Valdes, J., Holmes, D. S., & Dopson, M. (2011). Draft genome of the psychrotolerant acidophile *Acidithiobacillus ferrivorans* SS3. *Journal of Bacteriology*, 193(16), 4304–4305. <https://doi.org/10.1128/JB.05373-11>.
- Lin, J. T., & Stewart, V. (1997). Nitrate assimilation by bacteria. *Advances in Microbial Physiology*, 39, 1–30. [https://doi.org/10.1016/S0065-2911\(08\)60014-4](https://doi.org/10.1016/S0065-2911(08)60014-4).
- Lombard, V., Ramulu, H. G., Drula, E., Coutinho, P. M., & Henrissat, B. (2013). The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Research*, 42.
- López-Archilla, A. I., Marin, I., & Amils, R. (2001). Microbial community composition and ecology of an acidic aquatic environment: The Tinto River, Spain. *Microbial Ecology*, 41(1), 20–35. <https://doi.org/10.1007/s002480000044>.
- Mangold, S., Valdés, J., Holmes, D. S., & Dopson, M. (2011). Sulfur metabolism in the extreme acidophile *Acidithiobacillus caldus*. *Frontiers in Microbiology*, 2. <https://doi.org/10.3389/fmicb.2011.00017>.
- Mason, O. U., Hazen, T. C., Borglin, S., Chain, P. S. G., Dubinsky, E. A., Fortney, J. L., et al. (2012). Metagenome, metatranscriptome and single-cell sequencing reveal microbial response to Deepwater Horizon oil spill. *The ISME Journal*, 6(9), 1715–1727. <https://doi.org/10.1038/ismej.2012.59>.

- Mason, O. U., Scott, N. M., Gonzalez, A., Robbins-Pianka, A., Bælum, J., Kimbrel, J., et al. (2014). Metagenomics reveals sediment microbial community response to Deepwater Horizon oil spill. *The ISME Journal*, 8(7), 1464–1475. <https://doi.org/10.1038/ismej.2013.254>.
- McEwan, C. E. A., Gatherer, D., & McEwan, N. R. (1998). Nitrogen-fixing aerobic bacteria have higher genomic GC content than non-fixing species within the same genus. *Hereditas*, 128(2), 173–178. <https://doi.org/10.1111/j.1601-5223.1998.00173.x>.
- Melamud, V. S., Pivovarova, T. A., Tourova, T. P., Kolganova, T. V., Osipov, G. A., Lysenko, A. M., et al. (2003). *Sulfobacillus sibiricus* sp. nov., a new moderately thermophilic bacterium. *Microbiology*, 72(5), 605–612. <https://doi.org/10.1023/A:1026007620113>.
- Méndez-García, C., Peláez, A. I., Mesa, V., Sánchez, J., Golyschina, O. V., & Ferrer, M. (2015). Microbial diversity and metabolic networks in acid mine drainage habitats. *Frontiers in Microbiology*, 6(MAY). <https://doi.org/10.3389/fmicb.2015.00475>.
- Merrick, M., Javelle, A., Durand, A., Severi, E., Thornton, J., Avent, N. D., et al. (2006). The *Escherichia coli* AmtB protein as a model system for understanding ammonium transport by Amt and Rh proteins. *Transfusion Clinique et Biologique*, 13(1–2), 97–102. <https://doi.org/10.1016/j.tracbi.2006.02.015>.
- Meyer, B., & Kuever, J. (2007). Molecular analysis of the distribution and phylogeny of dissimilatory adenosine-5'-phosphosulfate reductase-encoding genes (aprBA) among sulfur-oxidizing. *Microbiology*, 153(10), 3478–3498. <https://doi.org/10.1099/mic.0.2007/008250-0>.
- Meyer, B., & Kuever, J. (2008). Homology modeling of dissimilatory APS reductases (AprBA) of sulfur-oxidizing and sulfate-reducing prokaryotes. *PLoS One*, 3, e1514. <https://doi.org/10.1371/journal.pone.0001514>.
- Mi, S., Song, J., Lin, J., Che, Y., Zheng, H., & Lin, J. (2011). Complete genome of *Leptospirillum ferriphilum* ML-04 provides insight into its physiology and environmental adaptation. *Journal of Microbiology*, 49(6), 890–901. <https://doi.org/10.1007/s12275-011-1099-9>.
- Mobley, H. L. T., & Hausinger, R. P. (1989). Microbial ureases: Significance, regulation, and molecular characterization. *Microbiological Reviews*, 53(1), 85–108. <https://doi.org/10.1128/mmbr.53.1.85-108.1989>.
- Mosier, A. C., Miller, C. S., Frischkorn, K. R., Ohm, R. A., Li, Z., LaButti, K., et al. (2016). Fungi contribute critical but spatially varying roles in nitrogen and carbon cycling in acid mine drainage. *Frontiers in Microbiology*, 7. <https://doi.org/10.3389/fmicb.2016.00238>.
- Musto, H., Naya, H., Zavala, A., Romero, H., Alvarez-Valín, F., & Bernardi, G. (2004). Correlations between genomic GC levels and optimal growth temperatures in prokaryotes. *FEBS Letters*, 573(1–3), 73–77. <https://doi.org/10.1016/j.febslet.2004.07.056>.
- Musto, H., Naya, H., Zavala, A., Romero, H., Alvarez-Valín, F., & Bernardi, G. (2006). Genomic GC level, optimal growth temperature, and genome size in prokaryotes. *Biochemical and Biophysical Research Communications*, 347(1), 1–3. <https://doi.org/10.1016/j.bbrc.2006.06.054>.
- Naya, H., Romero, H., Zavala, A., Alvarez, B., & Musto, H. (2002). Aerobiosis increases the genomic guanine plus cytosine content (GC%) in prokaryotes. *Journal of Molecular Evolution*, 55(3), 260–264. <https://doi.org/10.1007/s00239-002-2323-3>.
- Ogawa, K. I., Akagawa, E., Yamane, K., Sun, Z. W., LaCelle, M., Zuber, P., et al. (1995). The nasB operon and nasA gene are required for nitrate/nitrite assimilation in *Bacillus subtilis*. *Journal of Bacteriology*, 177(5), 1409–1413. <https://doi.org/10.1128/jb.177.5.1409-1413.1995>.
- Parro, V., Moreno-Paz, M., & González-Toril, E. (2007). Analysis of environmental transcriptomes by DNA microarrays. *Environmental Microbiology*, 9(2), 453–464. <https://doi.org/10.1111/j.1462-2920.2006.01162.x>.
- Price, G. D., Woodger, F. J., Badger, M. R., Howitt, S. M., & Tucker, L. (2004). Identification of a SulP-type bicarbonate transporter in marine cyanobacteria. *Proceedings of the National Academy of Sciences of the United States of America*, 101(52), 18228–18233. <https://doi.org/10.1073/pnas.0405211101>.
- Prosser, J. I., Bohannan, B. J. M., Curtis, T. P., Ellis, R. J., Firestone, M. K., Freckleton, R. P., et al. (2007). The role of ecological theory in microbial ecology. *Nature Reviews Microbiology*, 5(5), 384–392. <https://doi.org/10.1038/nrmicro1643>.
- Quatrini, R., Appia-Ayme, C., Denis, Y., Jedlicki, E., Holmes, D. S., & Bonnefoy, V. (2009). Extending the models for iron and sulfur oxidation in the extreme Acidophile *Acidithiobacillus ferrooxidans*. *BMC Genomics*, 10, 394. <https://doi.org/10.1186/1471-2164-10-394>.
- Rawlings, D. E. (2005). Characteristics and adaptability of iron-and sulfur-oxidizing microorganisms used for the recovery of metals from minerals and their concentrates. *Microbial Cell Factories*, 4.

- Richter, M., & Rosselló-Móra, R. (2009). Shifting the genomic gold standard for the prokaryotic species definition. *Proceedings of the National Academy of Sciences of the United States of America*, 106(45), 19126–19131. <https://doi.org/10.1073/pnas.0906412106>.
- Riesenfeld, C. S., Schloss, P. D., & Handelsman, J. (2004). Metagenomics: Genomic analysis of microbial communities. *Annual Review of Genetics*, 38, 525–552. <https://doi.org/10.1146/annurev.genet.38.072902.091216>.
- Rohwerder, T., Gehrke, T., Kinzler, K., & Sand, W. (2003). Bioleaching review part A: Progress in bioleaching: Fundamentals and mechanisms of bacterial metal sulfide oxidation. *Applied Microbiology and Biotechnology*, 63, 239–248. <https://doi.org/10.1007/s00253-003-1448-7>.
- Rohwerder, T., & Sand, W. (2007). Oxidation of inorganic sulfur compounds in acidophilic prokaryotes. *Engineering in Life Sciences*, 7(4), 301–309. <https://doi.org/10.1002/elsc.200720204>.
- Rosenthal, E., & Leustek, T. (1995). A multifunctional *Urechis caupo* protein, PAPS synthetase, has both ATP sulfurylase and APS kinase activities. *Gene*, 165(2), 243–248. [https://doi.org/10.1016/0378-1119\(95\)00450-K](https://doi.org/10.1016/0378-1119(95)00450-K).
- Sabater, S., Buchaca, T., Cambra, J., Catalan, J., Guasch, H., Ivorra, N., et al. (2003). Structure and function of benthic algal communities in an extremely acid river. *Journal of Phycology*, 39(3), 481–489. <https://doi.org/10.1046/j.1529-8817.2003.02104.x>.
- Sánchez-Andrea, I., Rodríguez, N., Amils, R., & Sanz, J. L. (2011). Microbial diversity in anaerobic sediments at Río Tinto, a naturally acidic environment with a high heavy metal content. *Applied and Environmental Microbiology*, 77(17), 6085–6093. <https://doi.org/10.1128/AEM.00654-11>.
- Schippers, A., Breuker, A., Blazejak, A., Bosecker, K., Kock, D., & Wright, T. L. (2010). The biogeochemistry and microbiology of sulfidic mine waste and bioleaching dumps and heaps, and novel Fe(II)-oxidizing bacteria. *Hydrometallurgy*, 104(3–4), 342–350. <https://doi.org/10.1016/j.hydromet.2010.01.012>.
- Schippers, A., & Sand, W. (1999). Bacterial leaching of metal sulfides proceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur. *Applied and Environmental Microbiology*, 65(1), 319–321. <https://doi.org/10.1128/aem.65.1.319-321.1999>.
- Schloss, P. D., & Handelsman, J. (2005). Metagenomics for studying unculturable microorganisms: Cutting the Gordian knot. *Genome Biology*, 6(8). <https://doi.org/10.1186/gb-2005-6-8-229>.
- Shively, J. M., Van Keulen, G., & Meijer, W. G. (1998). Something from almost nothing: Carbon dioxide fixation in chemoautotrophs. *Annual Review of Microbiology*, 52, 191–230. <https://doi.org/10.1146/annurev.micro.52.1.191>.
- So, A. K. C., Espie, G. S., Williams, E. B., Shively, J. M., Heinhorst, S., & Cannon, G. C. (2004). A novel evolutionary lineage of carbonic anhydrase (c class) is a component of the carboxysome shell. *Journal of Bacteriology*, 186(3), 623–630. <https://doi.org/10.1128/JB.186.3.623-630.2004>.
- Sogin, M. L., Morrison, H. G., Huber, J. A., Welch, D. M., Huse, S. M., Neal, P. R., et al. (2006). Microbial diversity in the deep sea and the underexplored rare biosphere. *Proceedings of the National Academy of Sciences of the United States of America*, 103(32), 12115–12120. <https://doi.org/10.1073/pnas.0605127103>.
- Streit, W. R., & Schmitz, R. A. (2004). Metagenomics - the key to the uncultured microbes. *Current Opinion in Microbiology*, 7(5), 492–498. <https://doi.org/10.1016/j.mib.2004.08.002>.
- Summers, Z. M., Fogarty, H. E., Leang, C., Franks, A. E., Malvankar, N. S., & Lovley, D. R. (2010). Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria. *Science*, 330(6009), 1413–1415. <https://doi.org/10.1126/science.1196526>.
- Talla, E., Hedrich, S., Mangenot, S., Ji, B., Johnson, D. B., Barbe, V., et al. (2014). Insights into the pathways of iron- and sulfur-oxidation, and biofilm formation from the chemolithotrophic acidophile *Acidithiobacillus ferrooxidans* CF27. *Research in Microbiology*, 165(9), 753–760. <https://doi.org/10.1016/j.resmic.2014.08.002>.
- Touati, D. (2000). Iron and oxidative stress in bacteria. *Archives of Biochemistry and Biophysics*, 373(1), 1–6. <https://doi.org/10.1006/abbi.1999.1518>.
- Travisany, D., Cortés, M. P., Latorre, M., Di Genova, A., Budinich, M., Bobadilla-Fazzini, R. A., et al. (2014). A new genome of *Acidithiobacillus thiooxidans* provides insights into adaptation to a bioleaching environment. *Research in Microbiology*, 165(9), 743–752. <https://doi.org/10.1016/j.resmic.2014.08.004>.
- Tripp, B. C., Smith, K., & Ferry, J. G. (2001). Carbonic anhydrase: New insights for an ancient enzyme. *Journal of Biological Chemistry*, 276(52), 48615–48618. <https://doi.org/10.1074/jbc.R100045200>.
- Tyson, G. W., Chapman, J., Hugenholtz, P., Allen, E. E., Ram, R. J., Richardson, P. M., et al. (2004). Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature*, 428(6978), 37–43. <https://doi.org/10.1038/nature02340>.

- Ullrich, S. R., González, C., Poehlein, A., Tischler, J. S., Daniel, R., Schlömann, M., et al. (2016). Gene loss and horizontal gene transfer contributed to the genome evolution of the extreme acidophile *Ferroplasma*. *Frontiers in Microbiology*, 7(MAY). <https://doi.org/10.3389/fmicb.2016.00797>.
- Ullrich, S. R., Poehlein, A., Tischler, J. S., González, C., Ossandon, F. J., Daniel, R., et al. (2016). Genome analysis of the biotechnologically relevant acidophilic iron oxidising strain JA12 indicates phylogenetic and metabolic diversity within the novel genus *Ferroplasma*. *PLoS One*, 11(1). <https://doi.org/10.1371/journal.pone.0146832>.
- Urich, T., Bandejas, T. M., Leal, S. S., Rachel, R., Albrecht, T., Zimmermann, P., et al. (2004). The sulphur oxygenase reductase from *Acidithiobacillus ferrooxidans* is a multimeric protein containing a low-potential mononuclear non-haem iron centre. *Biochemical Journal*, 381(1), 137–146. <https://doi.org/10.1042/BJ20040003>.
- Valdes, J., Ossandon, F., Quatrini, R., Dopson, M., & Holmes, D. S. (2011). Draft genome sequence of the extremely acidophilic biomining bacterium *Acidithiobacillus thiooxidans* ATCC 19377 provides insights into the evolution of the *Acidithiobacillus* genus. *Journal of Bacteriology*, 193(24), 7003–7004. <https://doi.org/10.1128/JB.06281-11>.
- Valdés, J., Pedroso, I., Quatrini, R., Dodson, R. J., Tettelin, H., Blake, R., et al. (2008). *Acidithiobacillus ferrooxidans* metabolism: From genome sequence to industrial applications. *BMC Genomics*, 9. <https://doi.org/10.1186/1471-2164-9-597>.
- Valdés, J., Pedroso, I., Quatrini, R., & Holmes, D. S. (2008). Comparative genome analysis of *Acidithiobacillus ferrooxidans*, *A. thiooxidans* and *A. caldus*: Insights into their metabolism and ecophysiology. *Hydrometallurgy*, 94(1–4), 180–184. <https://doi.org/10.1016/j.hydromet.2008.05.039>.
- Valdes, J., Quatrini, R., Hallberg, K., Dopson, M., Valenzuela, P. D. T., & Holmes, D. S. (2009). Draft genome sequence of the extremely acidophilic bacterium *Acidithiobacillus caldus* ATCC 51756 reveals metabolic versatility in the genus *Acidithiobacillus*. *Journal of Bacteriology*, 191(18), 5877–5878. <https://doi.org/10.1128/JB.00843-09>.
- Watling, H. R. (2006). The bioleaching of sulphide minerals with emphasis on copper sulphides - a review. *Hydrometallurgy*, 84(1–2), 81–108. <https://doi.org/10.1016/j.hydromet.2006.05.001>.
- Weber, K. A., Achenbach, L. A., & Coates, J. D. (2006). Microorganisms pumping iron: Anaerobic microbial iron oxidation and reduction. *Nature Reviews Microbiology*, 4(10), 752–764. <https://doi.org/10.1038/nrmicro1490>.
- Williams, K. P., & Kelly, D. P. (2013). Proposal for a new class within the phylum Proteobacteria, *Acidithiobacillia* classis nov., with the type order Acidithiobacillales, and emended description of the class Gammaproteobacteria. *International Journal of Systematic and Evolutionary Microbiology*, 63(8), 2901–2906. <https://doi.org/10.1099/ijs.0.049270-0>.
- Wintermute, E. H., & Silver, P. A. (2010). Emergent cooperation in microbial metabolism. *Molecular Systems Biology*, 6. <https://doi.org/10.1038/msb.2010.66>.
- Wood, A. P., Aurikko, J. P., & Kelly, D. P. (2004). A challenge for 21st century molecular biology and biochemistry: What are the causes of obligate autotrophy and methanotrophy? *FEMS Microbiology Reviews*, 28(3), 335–352. <https://doi.org/10.1016/j.femsre.2003.12.001>.
- Wu, X., Holmfeldt, K., Hubalek, V., Lundin, D., Åström, M., Bertilsson, S., et al. (2016). Microbial metagenomes from three aquifers in the Fennoscandian shield terrestrial deep biosphere reveal metabolic partitioning among populations. *The ISME Journal*, 10(5), 1192–1203. <https://doi.org/10.1038/ismej.2015.185>.
- Xiao, Y., Liu, X., Ma, L., Liang, Y., Niu, J., & Gu, Y. (2016). Microbial communities from different subsystems in biological heap leaching system play different roles in iron and sulfur metabolisms. *Applied Microbiology and Biotechnology*, 1–10. <https://doi.org/10.1007/s00253-016-7537-1>.
- Yan, L., Zhang, S., Wang, W., Hu, H., Wang, Y., Yu, G., et al. (2015). Draft genome sequence of *Acidithiobacillus ferrooxidans* YQH-1. *Genomics Data*, 6, 269–270. <https://doi.org/10.1016/j.gdata.2015.10.009>.
- Yelton, A. P., Comolli, L. R., Justice, N. B., Castelle, C., Denef, V. J., Thomas, B. C., et al. (2013). Comparative genomics in acid mine drainage biofilm communities reveals metabolic and structural differentiation of co-occurring archaea. *BMC Genomics*, 14(1). <https://doi.org/10.1186/1471-2164-14-485>.
- Yin, H., Niu, J., Ren, Y., Cong, J., Zhang, X., Fan, F., et al. (2015). An integrated insight into the response of sedimentary microbial communities to heavy metal contamination. *Scientific Reports*, 5. <https://doi.org/10.1038/srep14266>.
- Yin, H., Qiu, G., Wu, L., Xie, M., Zhou, J., Dai, Z., et al. (2008). Microbial community diversity and changes associated with a mine drainage gradient at the Dexing copper mine, China. *Aquatic Microbial Ecology*, 51(1), 67–76. <https://doi.org/10.3354/ame01172>.

- Yin, H., Zhang, X., Liang, Y., Xiao, Y., Niu, J., & Liu, X. (2014). Draft genome sequence of the extremophile *Acidithiobacillus thiooxidans* A01, isolated from the wastewater of a coal dump. *Genome Announcements*, 2, e214–e222. <https://doi.org/10.1128/genomeA.00222-14>.
- Yin, H., Zhang, X., Li, X., He, Z., Liang, Y., Guo, X., et al. (2014). Whole-genome sequencing reveals novel insights into sulfur oxidation in the extremophile *Acidithiobacillus thiooxidans*. *BMC Microbiology*, 14(1). <https://doi.org/10.1186/1471-2180-14-179>.
- Yoshizawa, Y., Toyoda, K., Arai, H., Ishii, M., & Igarashi, Y. (2004). CO₂-responsive expression and gene organization of three ribulose-1,5-bisphosphate carboxylase/oxygenase enzymes and carboxysomes in *Hydrogenovibrio marinus* strain MH-110. *Journal of Bacteriology*, 186(17), 5685–5691. <https://doi.org/10.1128/JB.186.17.5685-5691.2004>.
- You, X. Y., Guo, X., Zheng, H. J., Zhang, M. J., Liu, L. J., & Zhu, Y. Q. (2011). Unraveling the *Acidithiobacillus caldus* complete genome and its central metabolisms for carbon assimilation. *Journal of Genetics and Genomics*, 38(6), 243–252. <https://doi.org/10.1016/j.jgg.2011.04.006>.
- Zhang, X., Feng, X., Tao, J., Ma, L., Xiao, Y., Liang, Y., et al. (2016). Comparative genomics of the extreme *Acidophile acidithiobacillus thiooxidans* reveals intraspecific divergence and niche adaptation. *International Journal of Molecular Sciences*, 17(8). <https://doi.org/10.3390/ijms17081355>.
- Zhang, X., Liu, X., He, Q., Dong, W., Zhang, X., Fan, F., et al. (2016). Gene turnover contributes to the evolutionary adaptation of *Acidithiobacillus caldus*: Insights from comparative genomics. *Frontiers in Microbiology*, 7, 1960. <https://doi.org/10.3389/fmicb.2016.01960>.
- Zhang, X., Liu, X., Liang, Y., Fan, F., Zhang, X., & Yin, H. (2016). Metabolic diversity and adaptive mechanisms of iron- and/or sulfur-oxidizing autotrophic acidophiles in extremely acidic environments. *Environmental Microbiology Reports*, 8(5), 738–751. <https://doi.org/10.1111/1758-2229.12435>.
- Zhang, X., Liu, X., Liang, Y., Guo, X., Xiao, Y., Ma, L., et al. (2017). Adaptive evolution of extreme acidophile *Sulfo- bacillus thermosulfidooxidans* potentially driven by horizontal gene transfer and gene loss. *Applied and Environmental Microbiology*, 83(7). <https://doi.org/10.1128/AEM.03098-16>.
- Zhang, X., Niu, J., Liang, Y., Liu, X., & Yin, H. (2016). Metagenome-scale analysis yields insights into the structure and function of microbial communities in a copper bioleaching heap. *BMC Genetics*, 17(1). <https://doi.org/10.1186/s12863-016-0330-4>.
- Zhang, X., She, S., Dong, W., Niu, J., Xiao, Y., Liang, Y., et al. (2016e). Comparative genomics unravels metabolic differences at the species and/or strain level and extremely acidic environmental adaptation of ten bacteria belonging to the genus *Acidithiobacillus*. *Systematic and Applied Microbiology*, 39(8), 493–502. <https://doi.org/10.1016/j.syapm.2016.08.007>.

This page intentionally left blank

Properties of chalcopyrite

2.1 Oxidation state of Cu, Fe, and S elements

The crystal structure of CuFeS_2 was initially determined by Burdick and Eills through X-rays in 1917; they named the mineral chalcopyrite (Burdick & Ellis, 1917). Chalcopyrite belongs to the tetragonal system. Its crystal structure is similar to that of ZnS . The c -parameter of chalcopyrite cell is about twice that of ZnS ; in other words, the chalcopyrite unit cell seems to be composed of two unit cells of sphalerite (Li et al., 2013).

A sphalerite unit cell consists of four Zn atoms and four S atoms, in which Zn atoms occupy the (0, 0, 0) position and S atoms occupy (0.25, 0.25, 0.25) (Fig. 2.1A). The bond length between the Zn atom and the S atom is 2.34 Å, and the distance between two identical Zn atoms or S atoms is 3.83 Å (Edelbro et al., 2003; Li et al., 2013). The chalcopyrite unit cell consists of four Cu atoms, four Fe atoms, and eight S atoms (Fig. 2.1B). Each half of the chalcopyrite unit cell in the c -axis direction is composed of two Cu atoms and two Fe atoms replacing four Zn atoms in the zinc unit cell. Each S atom is coordinated with a tetrahedron composed of two Cu atoms and two Fe atoms, and each metal atom (Cu and Fe) is coordinated with a tetrahedron composed of four S atoms (Li et al., 2013).

Chalcopyrite belongs to the I–III–VI₂ type semiconductors with tetrahedral coordination. The crystal structure space group of the chalcopyrite is I–42d. In the chalcopyrite unit cell, Cu is located at the fractional coordinates of (0, 0, 0) and (0, 0.5, 0.25), and S is at (0.2575, 0.25, 0.125) (Edelbro et al., 2003; Li et al., 2013). The Fe atom at (0, 0, 0.5) has α -spin and the Fe atom at (0, 0.5, 0.75) has β -spin, so the chalcopyrite is an antiferromagnetic structure at room temperature (De Oliveira & Duarte, 2010; Li et al., 2013; Rais et al., 2000). The unit cell parameters of chalcopyrite are $a = b = 5.289$ Å, $c = 10.423$ Å, $\alpha = \beta = \gamma = 90$ degrees. The Cu–S and Fe–S bond lengths are 2.302 and 2.257 Å (Edelbro et al., 2003; Hall & Stewart, 1973; Li et al., 2013), respectively. The distance between two S atoms in a chalcopyrite unit cell is 3.6850, 3.7408, and 3.7956 Å (Hall & Stewart, 1973), and the distance between metal atoms is $d_{\text{Cu-Fe}} = d_{\text{Cu-Cu}} = d_{\text{Fe-Fe}} = 3.71$ Å (Edelbro et al., 2003).

The oxidation state for copper and iron in chalcopyrite are debated. Chalcopyrite is most often considered to be in the $\text{Cu}^{\text{I}}\text{Fe}^{\text{III}}\text{S}_2$ (Boekema et al., 2004; De Oliveira et al., 2012; Goh et al., 2006; Li et al., 2013; Mikhlin et al., 2004) or $\text{Cu}^{\text{II}}\text{Fe}^{\text{II}}\text{S}_2$ (Mikhlin et al., 2005; Todd et al., 2003) valence state. The oxidation state for copper in the form of $\text{Cu}^{\text{I}}\text{Fe}^{\text{III}}\text{S}_2$ is

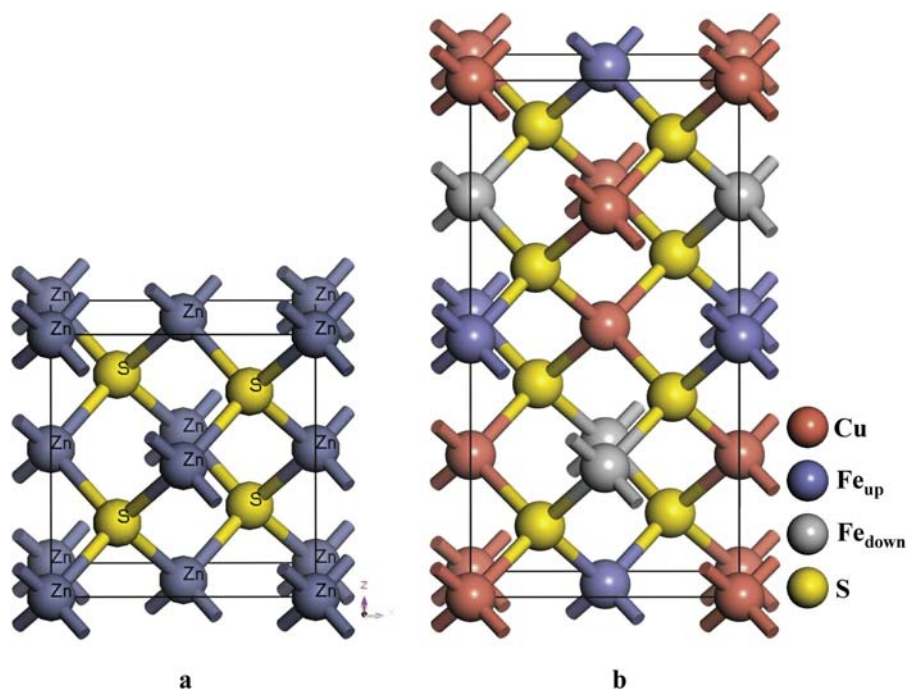


FIGURE 2.1 Schematic representation of (A) sphalerite and (B) chalcopyrite unit cell structure.

expected to Cu(I). Thus, the iron oxidation state would be Fe(III), whereas in the form of $\text{Cu}^{\text{II}}\text{Fe}^{\text{II}}\text{S}_2$, the oxidation state for copper in chalcopyrite is expected to Cu(II), and thus the iron oxidation state would be Fe(II).

Fig. 2.2 presents the X-ray photoelectron spectroscopy (XPS) of Cu 2p and Cu LMM for chalcopyrite after polishing and exposure to air for no more than a few minutes. The fitting results of peaks revealed that the Cu $2p_{3/2}$ peak of chalcopyrite is centered at 932.2 eV (Fig. 2.2A). The Cu LMM is centered at 568.9 eV (Fig. 2.2B). This value is in agreement

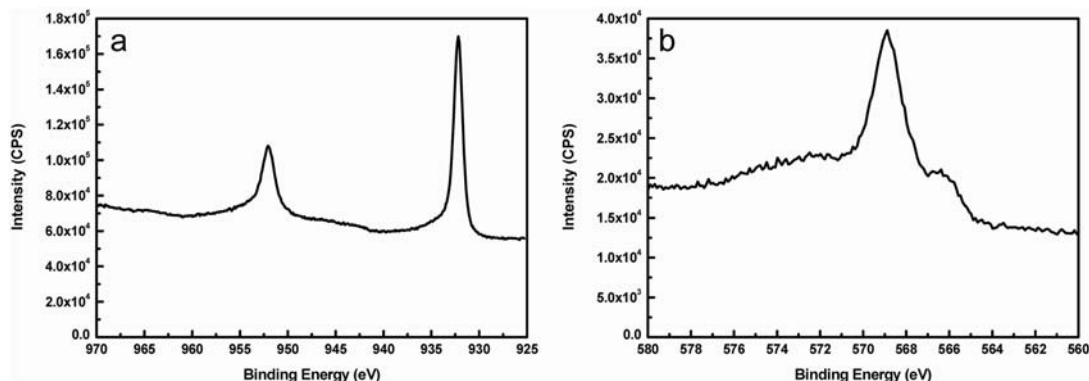


FIGURE 2.2 X-ray photoelectron spectroscopy spectra of (A) Cu 2p peak and (B) Cu LMM peak of chalcopyrite exposed to air for no more than a few minutes. Data from Wu, S., Yang, C., Qin, W., Jiao, F., Wang, J., & Zhang, Y. (2015). Sulfur composition on surface of chalcopyrite during its bioleaching at 50°C. Transactions of Nonferrous Metals Society of China, 25(12), 4110–4118. [https://doi.org/10.1016/S1003-6326\(15\)64062-6](https://doi.org/10.1016/S1003-6326(15)64062-6).

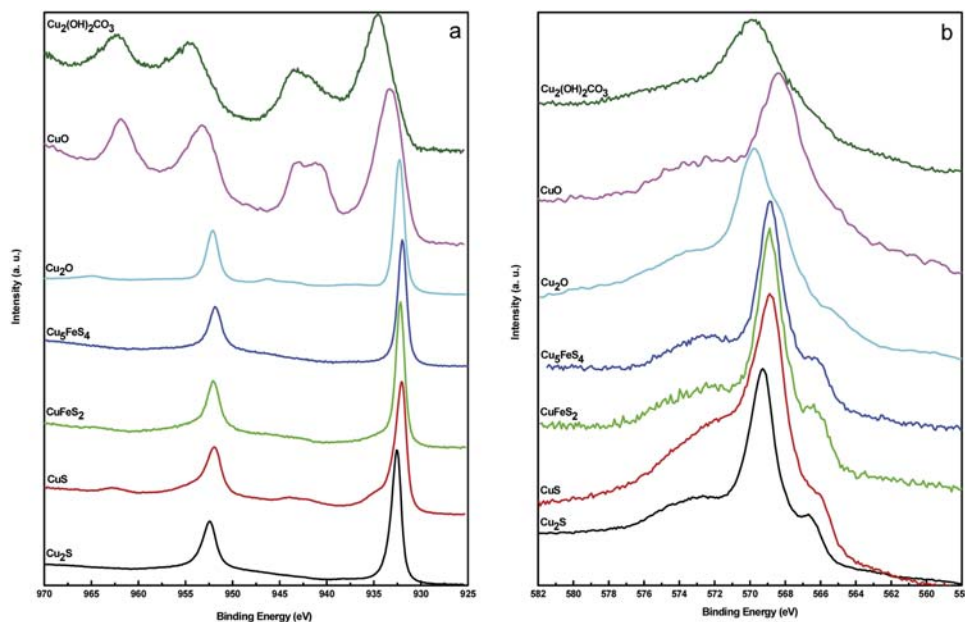


FIGURE 2.3 (A) Cu 2p and (B) Cu LMM peaks of Cu_2S , CuS , CuFeS_2 , Cu_5FeS_4 , Cu_2O , CuO , and $\text{Cu}_2(\text{OH})_2\text{CO}_3$.

with the literature (Ghahremaninezhad et al., 2013). The Cu 2p and Cu LMM spectra from the relatively pure chalcopyrite specimen are similar to those previously reported (Ghahremaninezhad et al., 2013; Goh et al., 2006).

To verify the oxidation state of Cu in chalcopyrite, the high-resolution Cu 2p and LMM spectra in Cu_2O , CuO , $\text{Cu}_2(\text{OH})_2\text{CO}_3$, Cu_2S , CuS , and Cu_5FeS_4 were also investigated. The high-resolution Cu 2p spectra of different minerals are shown in Fig. 2.3. The Cu $2p_{3/2}$ binding energy, full width at half maximum (FWHM), Cu LMM peak binding energy, and Auger parameters are listed in Table 2.1.

The Cu 2p spectrum of CuO and $\text{Cu}_2(\text{OH})_2\text{CO}_3$ appears as a strong satellite peak near 942 eV, whereas the Cu 2p spectrum of Cu_2O appears as only a single weak satellite peak near 945 eV. Similarly, no obvious strong satellite peaks are observed in the Cu 2p spectra of Cu_2S (Fig. 2.3A). Moreover, Cu $2p_{3/2}$ binding energy and Cu auger parameters of CuO and $\text{Cu}_2(\text{OH})_2\text{CO}_3$ are both higher than those in cuprous compounds such as Cu_2O and Cu_2S (Fig. 2.3 and Table 2.1). It is well-established in literature that there are two main XPS characteristics of Cu(II): a Cu $2p_{3/2}$ peak with a binding energy of 933.0–933.8 eV and the presence of a satellite peak features around the binding energy of 942 eV, whereas a lower Cu $2p_{3/2}$ binding energy of 931.8–933.1 eV and the absence of the satellite features are characteristics of Cu(I) and metal Cu (Chusuei et al., 1999; Ghahremaninezhad et al., 2013; Goh et al., 2006; Yin et al., 2005). In addition, there is no known report of an auger parameter less than 1850.5 eV for a Cu(II) compound (Goh et al., 2006). Fig. 2.3 and Table 2.1 show that the oxidation state for copper in chalcopyrite is expected to be Cu(I); therefore, the iron oxidation state would be Fe(III), and thus, chalcopyrite can be written as $\text{Cu}^{\text{I}}\text{Fe}^{\text{III}}\text{S}_2$.

However, Todd et al. (Todd et al., 2003) studied the Cu L-edges spectra of chalcopyrite, chalcocite, and covellite by X-ray absorption spectra. The results show that the Cu L-edges

TABLE 2.1 Binding energy (BE) and full width at half maximum values for X-ray photoelectron spectroscopy spectra of Cu $2p_{3/2}$ and Cu LMM peaks.

Mineral	Cu $2p_{3/2}$		Cu LMM	Auger parameter
	BE (eV)	Full width at half maximum	BE (eV)	BE (eV)
Cu ₂ (OH) ₂ CO ₃	934.6	3.9	569.7	1851.5
CuO	933.2	3.3	568.2	1851.6
Cu ₂ O	932.3	1.2	569.5	1849.5
Cu ₅ FeS ₄	932	1.1	568.8	1849.8
CuFeS ₂	932.2	1.1	568.9	1849.9
CuS	932.1	1.4	568.9	1849.8
Cu ₂ S	932.6	1.2	569.3	1849.9

of chalcopyrite have a strong absorption peak at 932.4 eV. The Cu L-edges of covellite have a strong absorption peak at 932.2 eV and a weak absorption peak at 935 eV. The Cu L-edges of chalcocite have a strong absorption peak only at 935 eV; no absorption peak is observed near 932 eV. The oxidation state of Cu in chalcocite is undoubtedly +1. Therefore, [Todd et al. \(2003\)](#) believed that Cu in covellite had two oxidation states: +2 (~932.2 eV) and +1 (~935 eV). Covellite can be written as Cu₂S·CuS₂ and the oxidation state for copper in chalcopyrite is also +2. It should be noted that if CuS were to be considered to be Cu₂S·CuS₂, with the tetrahedrally coordinated Cu in Cu₂S assumed to be Cu(I) and the trigonally coordinated Cu in CuS₂ assumed to be Cu(II) ([Goh, Buckley, & Lamb, 2006](#); [Goh, Buckley, Lamb, et al., 2006](#); [Todd et al., 2003](#)). Whereas the satellite peak around the binding energy of 942 eV should not be detected in our data; simultaneously, Ueda and van der Laan et al. believed that the copper in CuS₂ is monovalent ([Ueda et al., 2002](#); [van der Laan et al., 1992](#)). The two well-defined doublets indicated the presence of two different binding states of sulfur in the sample. Kundu et al. assumed that the covellite phase is represented by the formula Cu₃⁺(S₂)²⁻S⁻, in which two-thirds of the sulfur atoms form S₂ groups whereas one-third of the sulfur atoms are isolated ([Kundu, Hasegawa, Terabe, & Aono, 2008](#); [Kundu, Hasegawa, Terabe, Yamamoto, et al., 2008](#)). So, the copper in CuS should be monovalent ([Goh, Buckley, & Lamb, 2006](#); [Goh, Buckley, Lamb, et al., 2006](#); [Ueda et al., 2002](#); [van der Laan et al., 1992](#)).

The S 2p peaks also occur as doublets, S 2p_{3/2} and S 2p_{1/2}, as a result of spin–orbit splitting. The S 2p spectra were fitted using a 2:1 peak area ratio for S 2p_{3/2} to S 2p_{1/2} and 1.2 eV splitting. The peaks fitted to the spectra use a summed Gaussian–Lorentzian function to describe the convoluted Gaussian–Lorentzian line shapes of the peaks. The weighting used was 70% Gaussian and 30% Lorentzian. The background of the spectrum was obtained using the Shirley method.

[Fig. 2.4A](#) presents the S 2p peak of chalcopyrite consisting of three major doublets. The S 2p_{3/2} peaks are centered at 161.3, 161.9 and 162.8 eV, respectively. The prominent S 2p_{3/2} peak centered at 161.3 eV originates from S²⁻ in bulk chalcopyrite ([Ghahremaninezhad et al., 2013](#); [Nava et al., 2008](#)). The low-intensity S 2p_{3/2} peaks centered at 161.9 and 162.8 eV are assigned to S₂²⁻ ([Acres et al., 2010](#); [Klauber, 2003](#)) and S_n²⁻ ([Acres et al., 2010a, 2010b](#)), respectively. Klauber ([Klauber, 2003](#)) used XPS to study the composition of S on

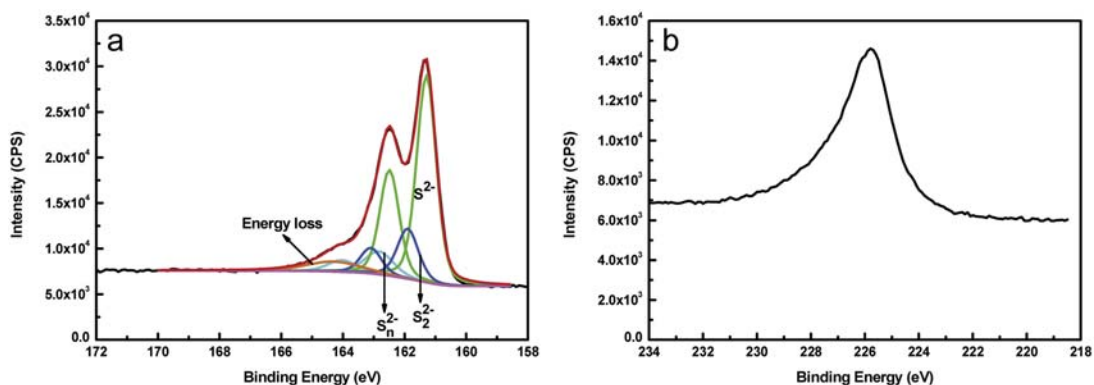
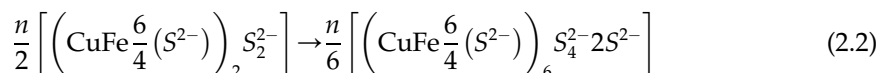
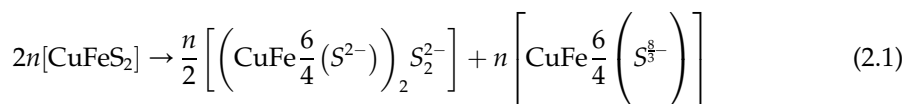


FIGURE 2.4 X-ray photoelectron spectroscopy spectrum of (A) S 2p peak and (B) S 2s peak of chalcopyrite exposed to air for no more than a few minutes. Data from Wu, S., Yang, C., Qin, W., Jiao, F., Wang, J., & Zhang, Y. (2015). Sulfur composition on surface of chalcopyrite during its bioleaching at 50°C. *Transactions of Nonferrous Metals Society of China*, 25(12), 4110–4118. [https://doi.org/10.1016/S1003-6326\(15\)64062-6](https://doi.org/10.1016/S1003-6326(15)64062-6).

the surface of chalcopyrite obtained by crushing in a nitrogen atmosphere. The results of XPS showed that S_2^{2-} was detected on the surface of chalcopyrite. The mechanism of formation of S_2^{2-} was explained by surface reconstruction, and a redox reaction model was also proposed. Similarly, Acres et al. (Acres et al., 2010) used synchrotron XPS to analyze the composition of S on the surface of chalcopyrite prepared in a nitrogen atmosphere. They found 11% of S_2^{2-} and 6% of S_n^{2-} on the surface of chalcopyrite. de Oliveira et al. (De Oliveira et al., 2012; De Oliveira & Duarte, 2010) applied density functional theory (DFT) to study chalcopyrite surface reconstruction. The results showed that S atoms in the first atomic layer of cleavage surfaces approach one another and form S_2^{2-} or S_4^{2-} . Moreover, S_2^{2-} is the precursor for forming S_4^{2-} . Li et al. (Li et al., 2014) further proposed chalcopyrite surface reconstruction mechanisms (Eqs. 2.1–2.2) based on a study by de Oliveira et al. (De Oliveira & Duarte, 2010; De Oliveira et al., 2012):



The S 2s photoelectron spectroscopy of chalcopyrite is shown in Fig. 2.4B. The binding energy of S 2s is 225.8 eV. The value of 225.6 eV is reported by Ghahremaninezhad et al. (Ghahremaninezhad et al., 2013). The S 2s binding energies of CuS, Cu₂S, and FeS₂ are 225.6, 225.3, and 226.1 eV, respectively; whereas the 2s binding energy of element S is 228.4 eV (Ghahremaninezhad et al., 2013).

In addition, a low-intensity 2p peak component at 164.3 eV can be ascribed to energy loss owing to S 3p → Fe 3d excitation (Acres et al., 2010; Klauber, 2003). To verify that the

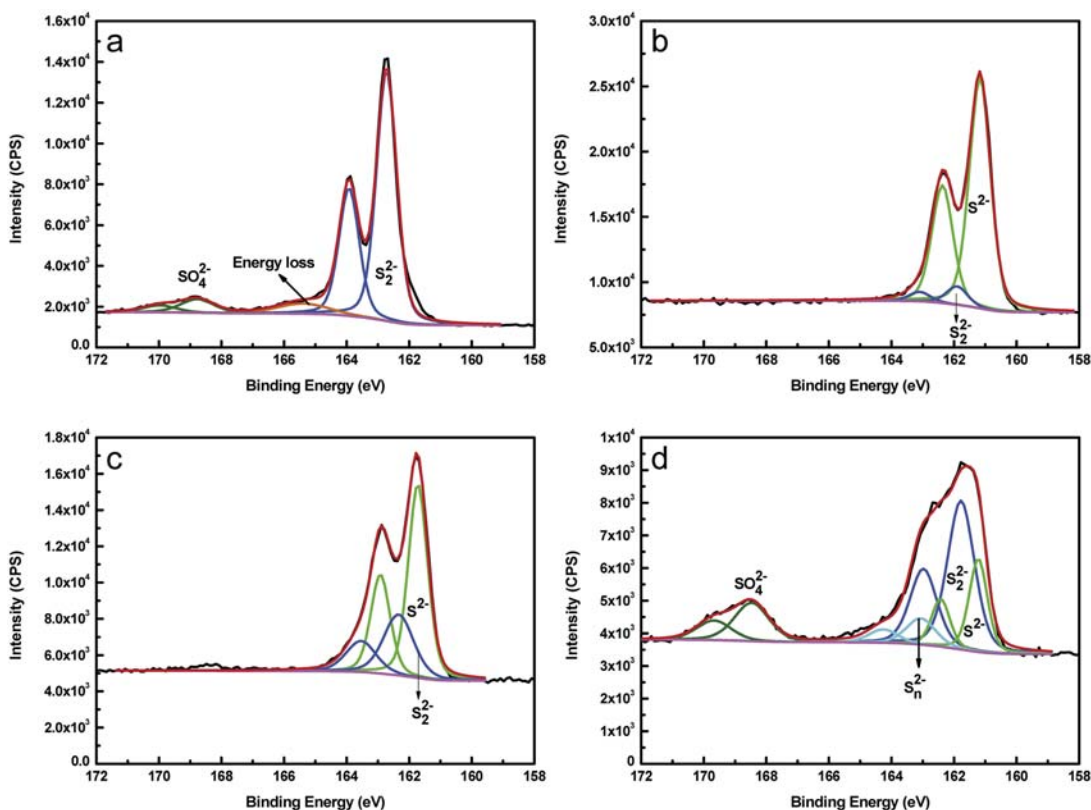


FIGURE 2.5 X-ray photoelectron spectroscopy spectrum of S 2p peak of (A) FeS_2 , (B) Cu_5FeS_4 , (C) Cu_2S , and (D) CuS . From Yang, C., Jiao, F., & Qin, W. (2018). Co-bioleaching of chalcopyrite and silver-bearing bornite in a mixed moderately thermophilic culture. *Minerals*, 8(1), 0–14. <https://doi.org/10.3390/min8010004>.

energy-loss peak is caused by $\text{S } 3p \rightarrow \text{Fe } 3d$ rather than $\text{S } 3p \rightarrow \text{Cu } 3d$ transfer, the S 2p photoelectron spectra of FeS_2 , Cu_5FeS_4 , Cu_2S , and CuS were further investigated. The fitting results of the S 2p peak are shown in Fig. 2.5. Fig. 2.5A shows that an energy-loss peak with $\text{FWHM} = 1.9 \text{ eV}$ is detected at the high binding energy edge, whereas there is no energy-loss peak in the S 2p photoelectron spectra of Cu_2S and CuS (Fig. 2.5B and C). The results further proved that the energy-loss peak centered at 164.3 eV in chalcopyrite was caused by $\text{S } 3p \rightarrow \text{Fe } 3d$ excitation. However, Cu_5FeS_4 also consisted of Cu, Fe, and S. No energy-loss peak was found in the fitted S 2p photoelectron spectra (Fig. 2.5D). Acres et al. (Acres et al, 2010a, 2010b) reported that an energy-loss peak centered at 163.9 eV was found in S 2p photoelectron spectra of bornite.

The high-resolution spectrum of Fe 2p for chalcopyrite exposed to air for no more than a few minutes is provided in Fig. 2.6A. The Fe 2p spectrum shows that even chalcopyrite exposed to air for only a few minutes was oxidized. The peak near 708 eV can be assigned to Fe–S (Ghahremaninezhad et al., 2013). The peak in the binding energy range of 710–712 eV can be assigned to Fe–O and Fe–OH (Li et al., 2014). This can be proved by

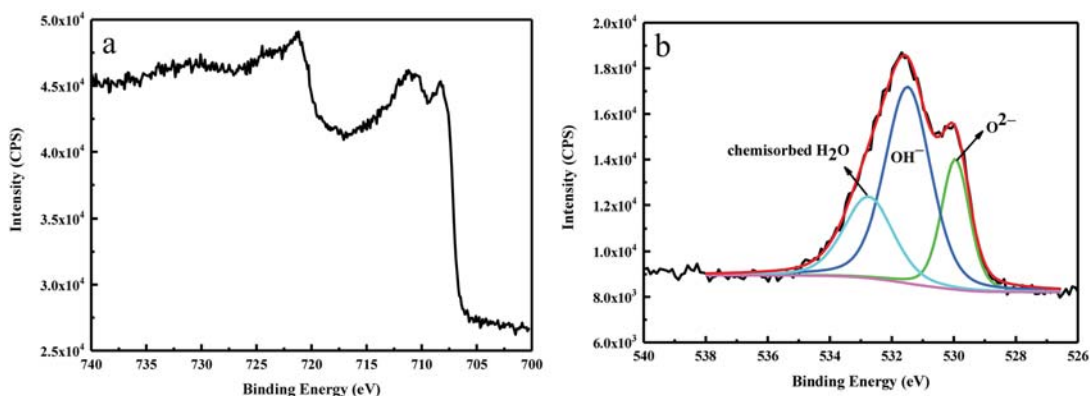


FIGURE 2.6 X-ray photoelectron spectroscopy spectrum of (A) Fe 2p peak and (B) O 1s peak of chalcopyrite after polishing and exposure to air for no more than a few minutes. From Wu, S., Yang, C., Qin, W., Jiao, F., Wang, J., & Zhang, Y. (2015). Sulfur composition on surface of chalcopyrite during its bioleaching at 50°C. *Transactions of Nonferrous Metals Society of China*, 25(12), 4110–4118. [https://doi.org/10.1016/S1003-6326\(15\)64062-6](https://doi.org/10.1016/S1003-6326(15)64062-6).

the O 1s spectrum, which can be fitted with O^{2-} , OH^- , and chemisorbed H_2O (Li et al., 2014) (Fig. 2.6B). Moreover, Goh et al. (Goh et al., 2006) suggested that the presence of O on the chalcopyrite surface was inconsistent with Fe–O compared with Cu–O species. According to Li et al. (Li et al., 2014), the formation of Fe–O occurs as a result of O_2 adsorption on Fe sites with subsequent dissociation, and the formation of Fe–OH is due to H_2O adsorption on Fe sites with subsequent dissociation. Furthermore, de Lima et al. (De Lima et al., 2011) reported that water molecules and the OH group absorb preferentially on iron atoms of reconstructed chalcopyrite (001) sulfur-terminated surfaces via the Fe–O bond. Similar outcomes were seen on a pyrite (FeS_2) surface. Water molecule adsorption on the FeS_2 surface occurs via an Fe–O bond (Chen et al., 2014; Stirling et al., 2003). Mishra and Osseoasare (Mishra & Osseo-Asare, 1988) stated that the opening step of pyrite oxidation in an aqueous solution is the adsorption OH group on iron sites of the pyrite surface. Therefore, O^{2-} , OH^- , and chemisorbed H_2O are most probably adsorbed on iron sites of the chalcopyrite surface. Moreover, a binding energy near 712.5 eV would most probably be a Cu LMM peak (Ghahremaninezhad et al., 2013; Harmer et al., 2005).

2.2 Electronic structure

An understanding of the electronic structure and surface properties of chalcopyrite can help us explain the leaching behavior of chalcopyrite and the mechanism of formation of disulfide and polysulfide from a microscopic level. Therefore, the electronic structure and surface properties of chalcopyrite were investigated by DFT. DFT calculations were performed using CASTEP. The generalized gradient approximation (GGA) for the exchange and correlation potential (XC) proposed by Perdew and Wang (PW91) was used (De Oliveira et al., 2012). The core electrons were described by ultrasoft pseudopotentials considering the follow valence configuration: Fe $3d^6 4s^2$, Cu $3d^{10} 4s^1$, and S $3s^2 3p^4$. Electron correlation effects are important for the electronic structure of transition-metal compounds; thus, we

TABLE 2.2 Convergence test results of energy cutoff.

Energy cutoff (eV)	Lattice parameters (Å)		$dE_{tot}/d\log(E_{cut})$	Final energy (eV)	Error (%)	
	$a = b$	c			$a = b$	c
300	5.35	10.542	-22.916079	-11,606.463113	1.25	1.2
320	5.324	10.477	-12.018541	-11,606.830257	0.76	0.57
340	5.32	10.448	-3.982405	-11,607.168968	0.69	0.3
360	5.306	10.434	-1.849401	-11,607.175514	0.42	0.16
380	5.305	10.418	-0.270471	-11,607.203774	0.39	0.01
400	5.301	10.418	-0.074255	-11,607.207517	0.32	0.01

used Hubbard U corrections for the band structure, electron density, and density of states calculation, but not for geometry optimization (Himmetoglu et al., 2014). In our calculations, we adopt the U values of 2.5 eV for Cu and Fe, which best fit the literature (0.53–0.6 eV) (Li et al., 2013) optical band gap values (Szpunar, 2012). To test how the selected pseudopotentials and energy cutoff would describe the known crystalline structure of CuFeS_2 , a geometry optimization of a crystalline model was performed, relaxing the cell parameters using a series of cutoff energies. The convergence tolerance of the total energies, maximum force, and maximum displacement were 1.0×10^{-5} eV/atom, 0.03 eV/Å, and 0.001 Å, respectively. The self-consistent field (SCF) convergence tolerance was 1.0×10^{-6} eV/atom. When the cut-off energy is 400 eV, the value of $dE_{tot}/d\log(E_{cut})$ is less than 0.1; and the cell parameters of the chalcopyrite are computed to be $a = b = 5.301$ Å and $c = 10.418$ Å, compared to the experimental value, the error less than 0.4% (Table 2.2). The XRD pattern obtained by the simulate calculation agrees well with the experimental values (Fig. 2.7), indicating that the crystal model and parameter settings are reasonable, and the calculated results have reference value.

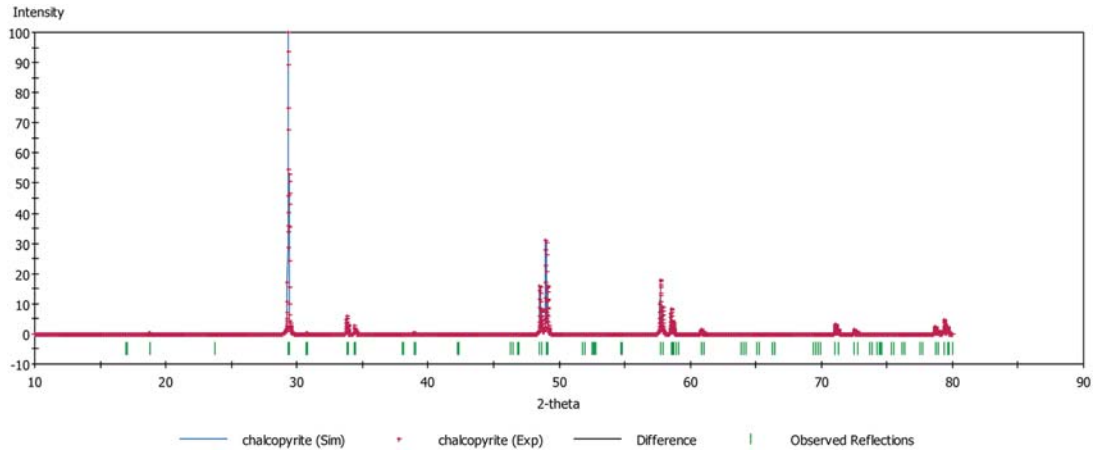


FIGURE 2.7 X-ray photoelectron spectroscopy pattern obtained from experiment (Exp) and simulation (Sim).

TABLE 2.3 Mulliken population of chalcopyrite.

Species	<i>s</i>	<i>p</i>	<i>d</i>	Total	Charge (e)
S	1.82	4.5	0	6.32	−0.32
Fe _{up}	0.49	0.64	6.4	7.53	0.47
Fe _{down}	0.49	0.64	6.4	7.53	0.47
Cu	0.53	0.51	9.79	10.83	0.17

The calculated Mulliken population of chalcopyrite is shown in Table 2.3. The calculation results show that Cu atoms and Fe atoms transfer 0.17 and 0.47 electrons to S atoms, respectively, and the electron transfer ratios of Cu and Fe are close to 1:3. Therefore, it is further proved that the oxidation states of Cu, Fe, and S in chalcopyrite are +1, +3, and −2, respectively.

The calculated band structure for the chalcopyrite is shown in Fig. 2.8. The band gap of the chalcopyrite is calculated to be 0.596 eV. This value is in agreement with the literature (Li et al., 2013). The total and partial density of states for the chalcopyrite are shown in Fig. 2.9. Combined with Figs. 2.8 and 2.9, it can be seen that the valence band in the range of −13.8 to −11.7 eV mainly consists of S 3s; the valence band in the range of −6.3 to −4.7 eV mainly consists of Fe 3d and S 3p, together with some Cu 3d. From −4.7 eV to Fermi level, it mainly consists of Cu 3d and S 3p, together with some Fe 3d. The conduction band in the range of 0–2.4 eV mainly consists of Fe 3d, together with some S 3p. From this analysis, we know that the conduction band of chalcopyrite mainly consists of Fe 3d empty orbitals, so Fe atoms can capture electrons and be reduced.

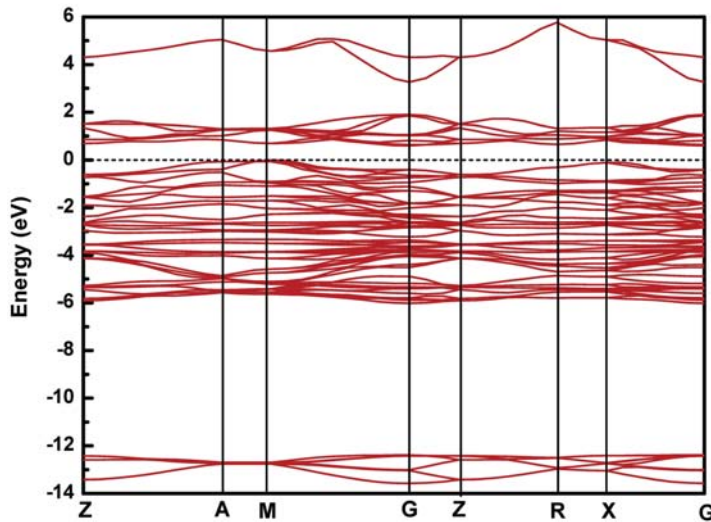


FIGURE 2.8 Calculated band structure for chalcopyrite. The zero value of energy has been set at the Fermi level.

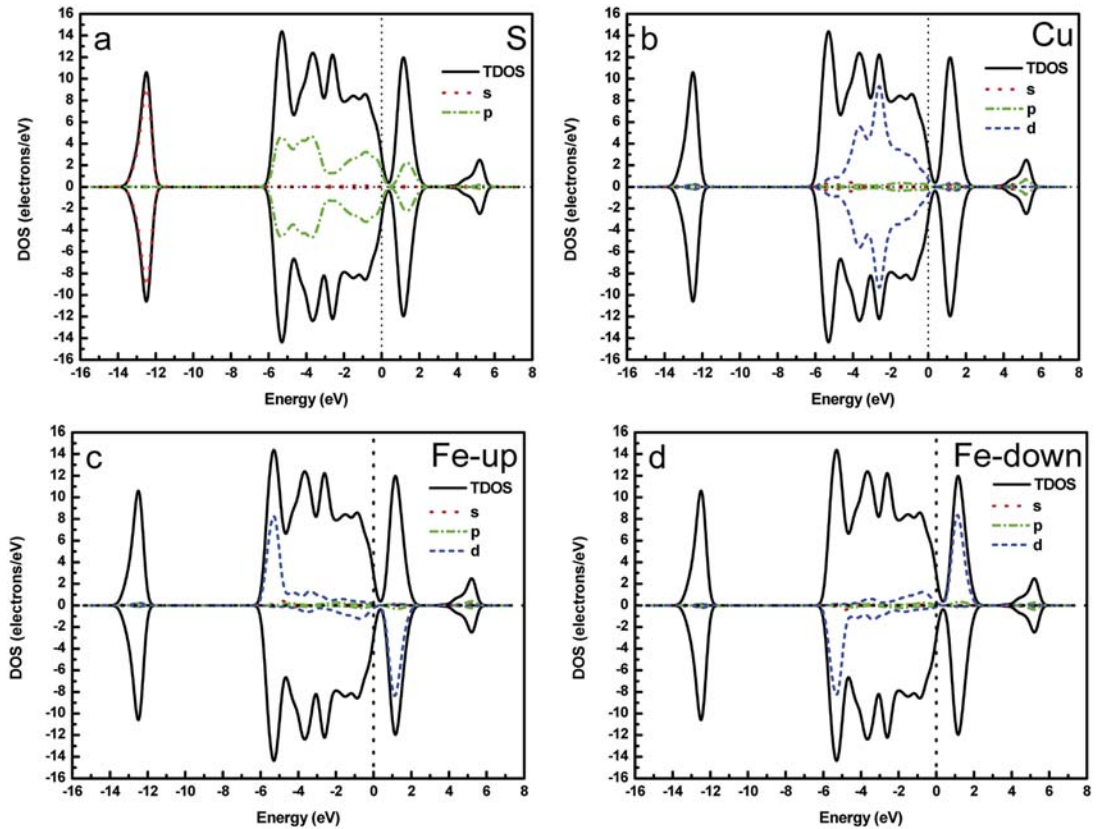


FIGURE 2.9 Total density of states (TDOS) and partial density of states (DOS).

2.3 Reconstruction of chalcopyrite surfaces

2.3.1 (0 0 1) surface

S_2^{2-} and S_n^{2-} were detected on the surface of chalcopyrite after polishing and exposure to air for no more than a few minutes. Furthermore, Klauber (Klauber, 2003) reported that S_2^{2-} formed on freshly fractured chalcopyrite surfaces in an inert atmosphere. Therefore, DFT calculations were also employed to understand the mechanisms of formation for S_2^{2-} and S_n^{2-} .

The surfaces were reconstructed using geometry optimization. The chalcopyrite stoichiometry (1:1:2) was fixed in all calculations. A vacuum of 15 Å along the *c* direction was established to avoid interactions between the top and bottom surfaces in the slab. We optimized the surface atomic layers (three atomic layers) whereas the other atomic layers were fixed in the bulk geometry. Chalcopyrite exhibits poor cleavage at the (112) plane; hence, fracture of the chalcopyrite exposes many differently oriented surfaces (De Oliveira et al., 2012). Here, we describe the reconstructed (001) and (112) surface of chalcopyrite.

The sulfur terminated surface and metal terminated surface for the (001) surface of chalcopyrite are represented by (001)-S and (001)-M, respectively. The unreconstructed (001)-S terminated surface has two S atoms in the first atomic layer. Each S atom is coordinated with one Cu atom and one Fe atom of the second layer, as shown in Fig. 2.10A and B. Two S atoms in the first atomic layer approach one another and form an S–S bond during the structural optimization, as shown in Fig. 2.10C and D. The S–S bond length is 2.16 Å, the distance between each S of the S–S bond and its coordinate Cu atom is 2.333 Å, and the distance between each S of the S–S bond and its coordinate Fe atom is 2.266 Å. The distance between S–S and S–Cu is consistent with the results calculated by de Oliveira and Duarte, whereas the S–Fe distance calculated by de Oliveira and Duarte is 2.32 Å (De Oliveira & Duarte, 2010).

The S–S bond length formed at the (001)-S terminated surface is close to the bond length of S_2^2 ; its bond length is 2.276 Å (De Oliveira & Duarte, 2010). To prove that the two S atoms in the (001)-S terminated surface reconstitute to form S_2^{2-} , the change in electron density before and after reconstruction of the (001)-S terminated surface was calculated. The calculation results are shown in Fig. 2.11. Before reconstitution, the electron density of the two S atoms in the (001)-S terminated surface did not overlap (Fig. 2.11A), but after relaxation and reconstruction, the electron densities of the two S atoms overlapped (Fig. 2.11B), indicating that S_2^{2-} formed through reconstruction of the (001)-S terminated surface.

The result of XPS and DFT calculation indicated that the oxidation states of Cu, Fe, and S in chalcopyrite are +1, +3, and –2, respectively, and chalcopyrite can be written as $Cu^I-Fe^{III}S_2$. To understand the mechanisms of formation for S_2^{2-} , the sum of local density of states (LDOS) on all S atoms in the first atomic layer and Fe, Cu atoms in the second atomic layer in both the unreconstructed and reconstructed (001)-S terminated surface was calculated (Fig. 2.12). Fig. 2.12A shows that the number of occupied electronic states on S atoms in the region of –1 to 0 eV decreased owing to the reconstruction, which means that the new S–S bond formed in an oxidative process. Fig. 2.12B shows that the number of occupied electronic states on Fe atoms in the region of –1 to 0 eV increased owing to the reconstruction, but similar behavior was not observed for the LDOS on the Cu atoms (Fig. 2.12C). These results suggest that electrons released with the formation of S_2^{2-} are transferred to Fe atoms.

The unreconstructed (001)-M terminated surface has four Cu atoms and one Fe atom in the first atomic layer, two S atoms in the second atomic layer, and two Cu atoms and two Fe in the third atomic layer, as shown in Fig. 2.13A and B. After geometry optimization, five metal atoms in the first atomic layer before reconstruction migrate downward, whereas four metal atoms in the third atomic layer before reconstruction migrate upward. The downward migrating metal atoms and the upward migrating metal atoms eventually occupy the same plane and form a structure similar to the Cu–Fe alloy. The bond length between metal atoms is 2.65–2.67 Å (Fig. 2.13C–D). The two S atoms of the second atomic layer before reconstruction migrate to the surface to become the first atomic layer and occupy the central position of the rectangle formed by the metal atoms (Fig. 2.13C and D). The distance between the first layer of S atoms and the second layer of metal atoms has an S–Fe bond length of 2.22 Å, whereas the S–Cu bond length is 2.27 and 2.32 Å.

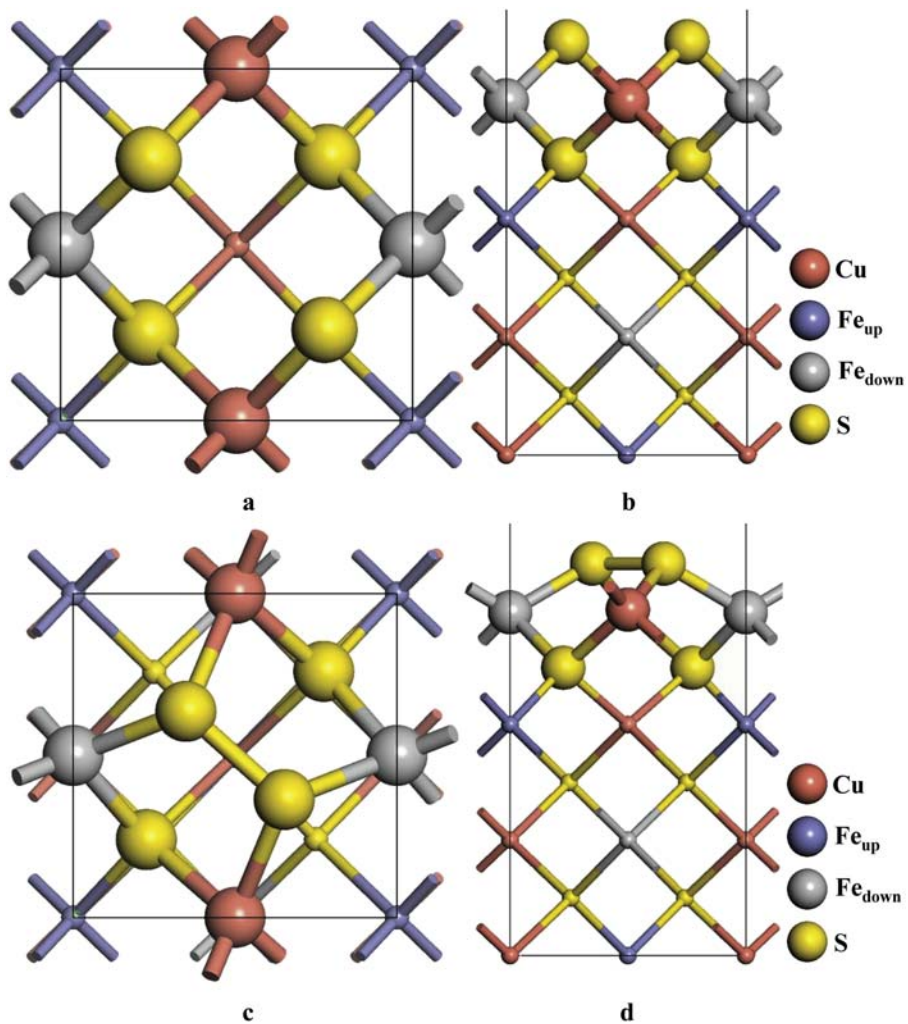


FIGURE 2.10 (A) Top and (B) side views of the unreconstructed (001)-S chalcopyrite surface. (C) Top and (D) side views of the reconstructed (001)-S chalcopyrite surface.

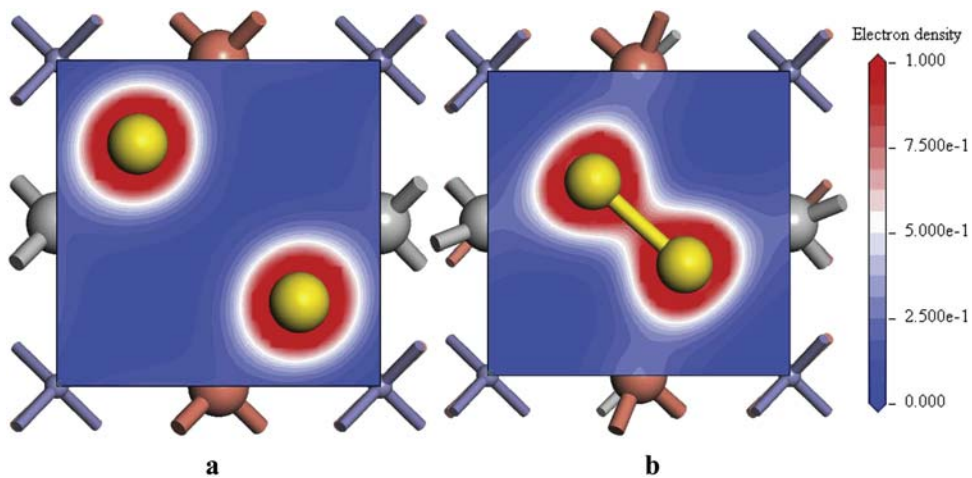


FIGURE 2.11 Electron density of the (A) unreconstructed and (B) reconstructed (001)-S chalcopyrite surface.

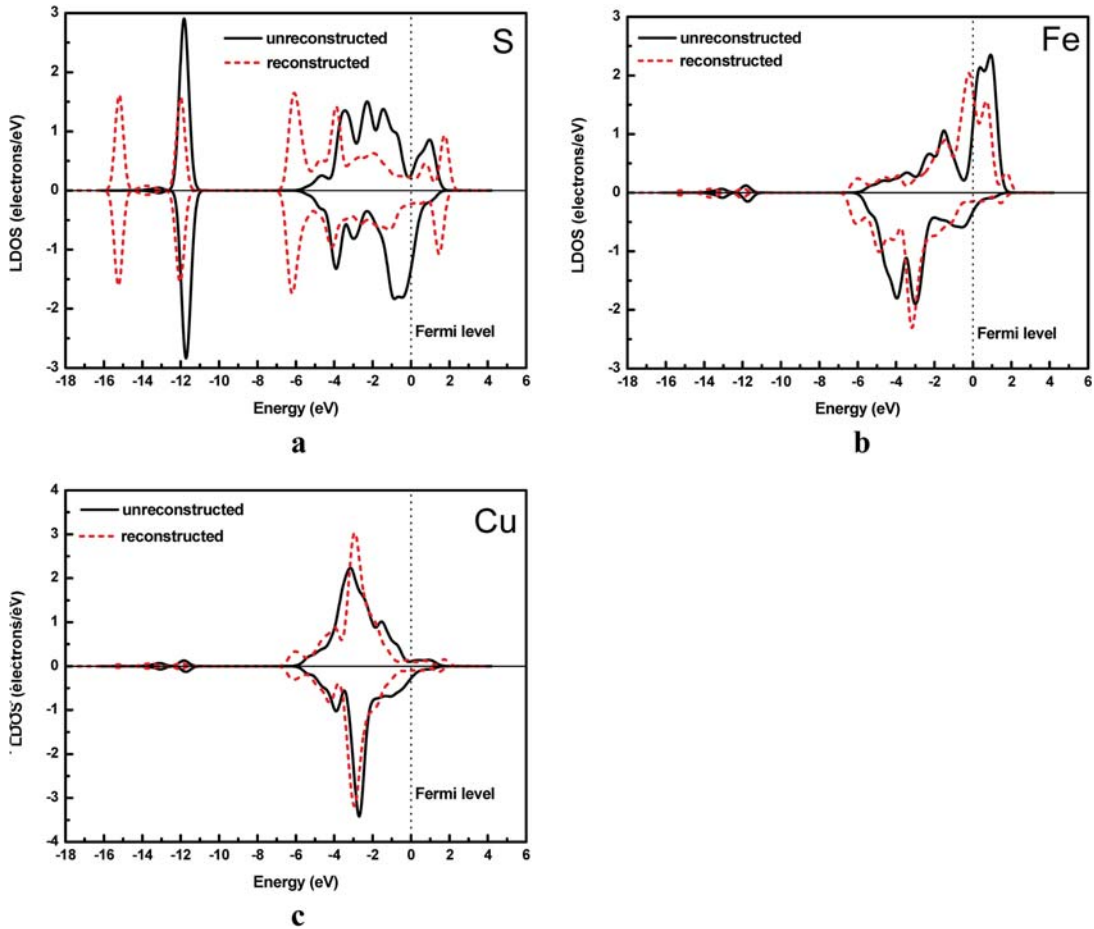


FIGURE 2.12 Density of states on (A) sulfur atoms, (B) iron atoms, and (C) copper atoms in reconstructed (001)-S chalcopyrite surface. *LDOS*, local density of states.

The change in electron density before and after the reconstruction of (001)-M terminated surface was calculated, the calculation results are shown in Fig. 2.14. Before reconstitution, the electron density of the first atomic layer and the third atomic layer in the (001)-M terminated surface mainly concentrated near the metal atoms and the electron density between the metal atoms did not overlap (Fig. 2.14A and B). However, after relaxation and reconstruction, the electron densities were distributed in the entire planar region (Fig. 2.14C), indicating that the (001)-M terminated surface was reconstructed and formed a Cu–Fe alloy structure. Before (001)-M terminated surface reconstruction, the electronic state of Cu and Fe was concentrated near -2.5 eV and -3 eV, respectively (Fig. 2.15); however, after reconstruction,

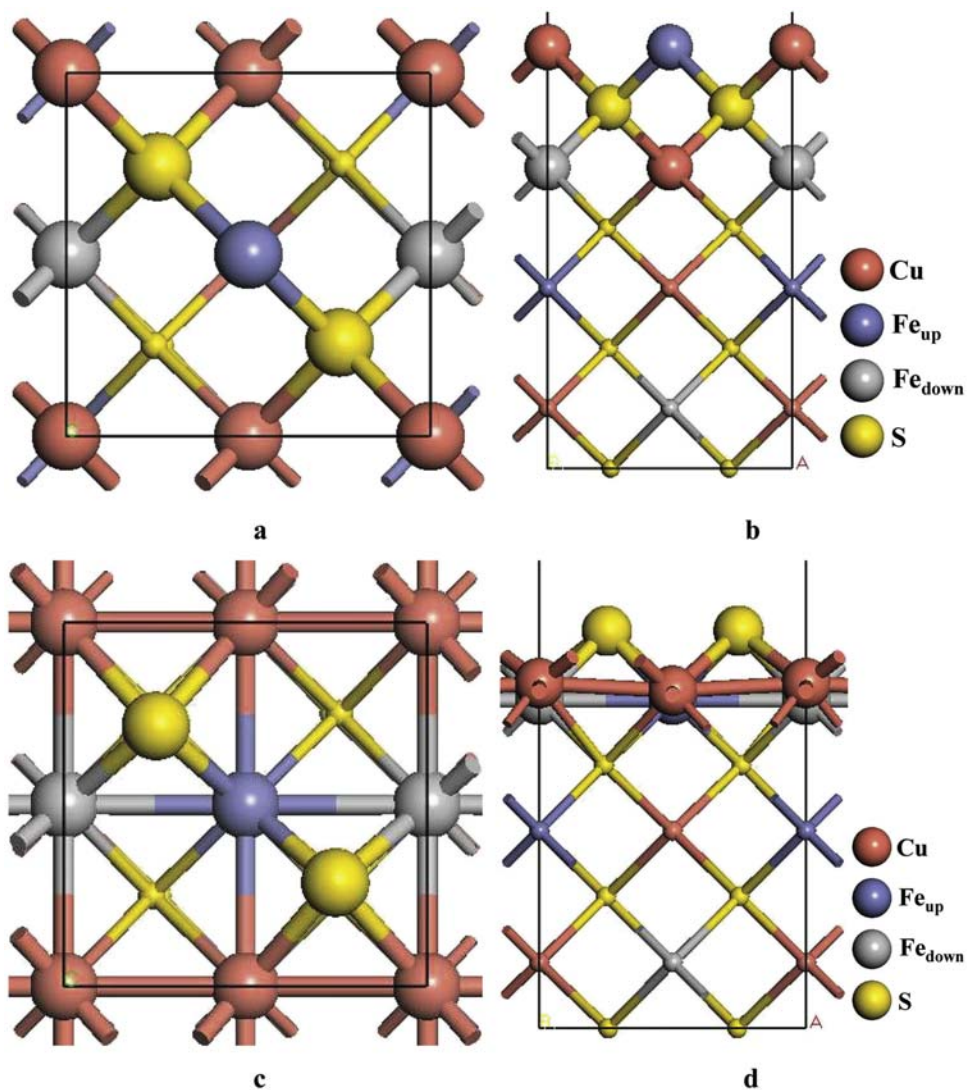


FIGURE 2.13 (A) Top and (B) side views of unreconstructed and reconstructed (001)-M chalcopyrite surface. (C) Top and (D) side views of reconstructed (001)-M chalcopyrite surface.

the electronic state was distributed to a wider energy region, proving the formation of a Cu–Fe alloy structure.

2.3.2 (1 1 2) surface

The sulfur terminated surface and metal terminated surface for the (112) surface of chalcopyrite are represented by (112)-S and (112)-M, respectively. The unreconstructed (112)-S

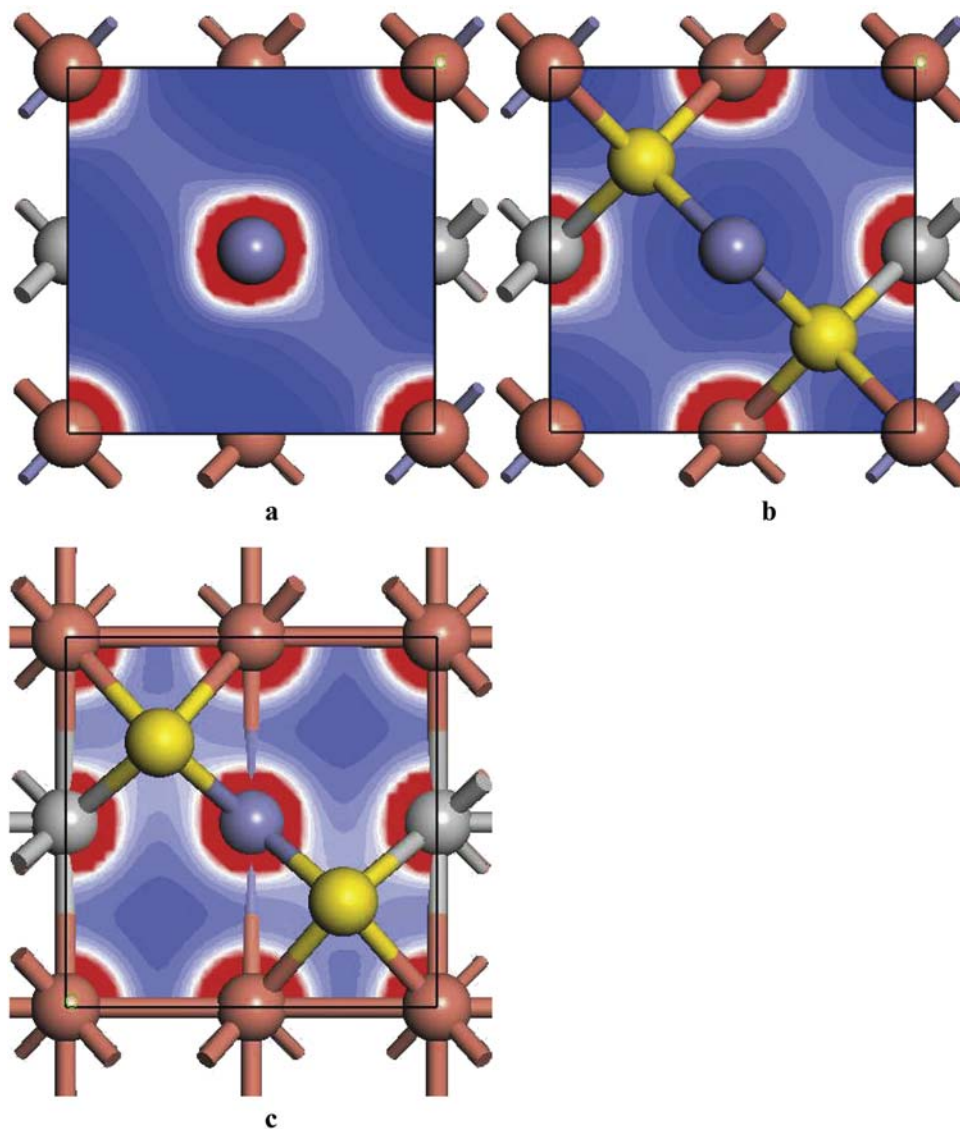


FIGURE 2.14 Electron density of unreconstructed (A) first atomic layer and (B) third atomic layer and (C) reconstructed (001)-M terminated surface of chalcopyrite.

terminated surface has four S atoms in the first atomic layer. Each S atom is coordinated with one Cu atom or one Fe atom of the second layer, as shown in Fig. 2.16A and B. The four S atoms in the first atomic layer approach one another and form a chain polysulfide of four S (S_4^{2-}) during structural optimization, as shown in Fig. 2.16C and D. The polysulfide is thought to be formed by the repolymerization of two disulfides.

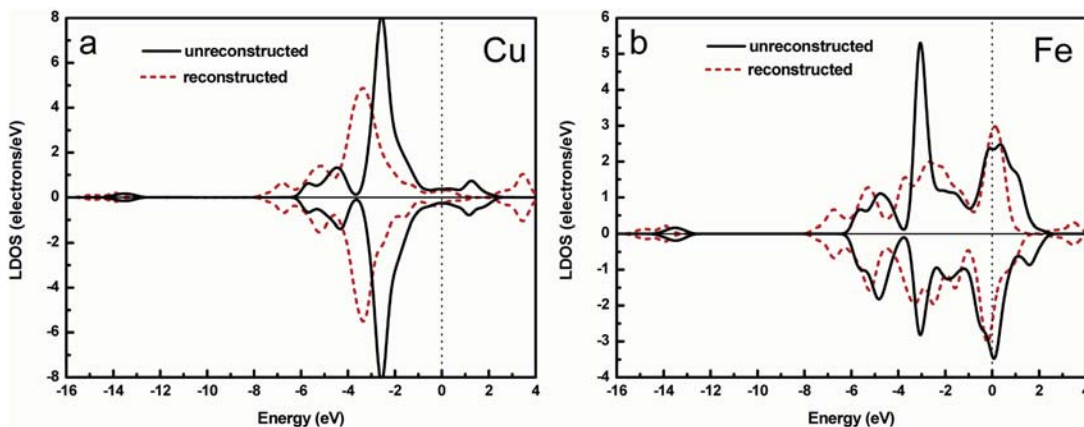


FIGURE 2.15 Density of states on sulfur atoms, iron atoms, and copper atoms in reconstructed (001)-M chalcopyrite surface. LDOS, local density of states.

Structural optimization based on DFT is a process of finding the lowest energy; that is, the lower the energy of the system, the more stable the optimized structure will be. As can be seen from the change in total energy during geometry optimization of the (112)-S terminated surface (Fig. 2.17), when the optimization reaches step 18, the total energy of the system sharply decreases and two disulfides are simultaneously formed at the (112)-S terminated surface. The S atom in the disulfide composed of one Fe atom and one Cu atom. As optimization continues, the energy of the system is further reduced and a stable structure is finally obtained. The two disulfides are further polymerized to form a chain polysulfide of four S atoms. Therefore, S_2^{2-} is the precursor to forming S_4^{2-} (De Oliveira et al., 2012).

The bond length of the S–S formed by the S coordinated with the Fe_{down} and Cu atom is 2.027 Å, and the corresponding bond length of the S– Fe_{down} and S–Cu is 2.280 and 2.351 Å, respectively; the bond length of the S–S formed by the S coordinated with the Fe_{up} and Cu atom is 2.019 Å; and the corresponding bond length of the S– Fe_{up} and S–Cu is 2.286 and 2.394 Å, respectively. The spin of Fe does not affect the formation of S_2^{2-} on the (112)-S terminated surface of chalcopyrite. The bond length of S–S formed by S composed of two Cu atoms is calculated to be 2.253 Å. To prove that the four S atoms in the (112)-S terminated surface reconstitute to form S_4^{2-} , the change in electron density before and after reconstruction of the (112)-S terminated surface was calculated. The calculation results are shown in Fig. 2.18. Before reconstitution, the electron density of two S atoms in the (112)-S terminated surface did not overlap (Fig. 2.18A), but after relaxation and reconstruction, the electron densities of the four S atoms overlapped (Fig. 2.11B), indicating that S_4^{2-} formed through reconstruction of the (112)-S terminated surface.

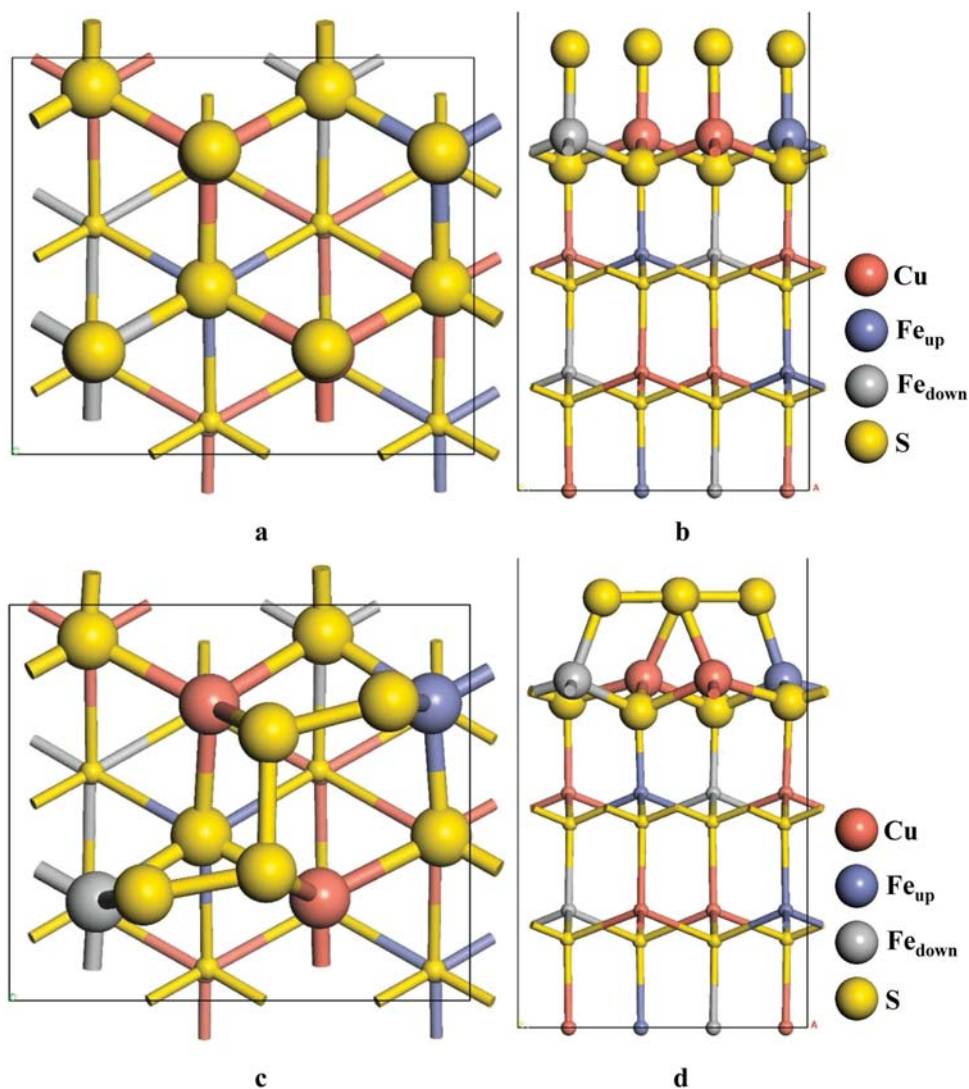


FIGURE 2.16 (A) Top and (B) side views of unreconstructed (112)-S chalcopyrite surface. (C) Top and (D) side views of reconstructed (112)-S chalcopyrite surface.

The sum of LDOS on all S atoms in the first atomic layer and Fe, Cu atoms in the second atomic layer in both unreconstructed and reconstructed (112)-S terminated surface was calculated (Fig. 2.19). Fig. 2.19A shows that the number of occupied electronic states on S atoms in the region of -1 to 0 eV decreased owing to the reconstruction, which means that the new

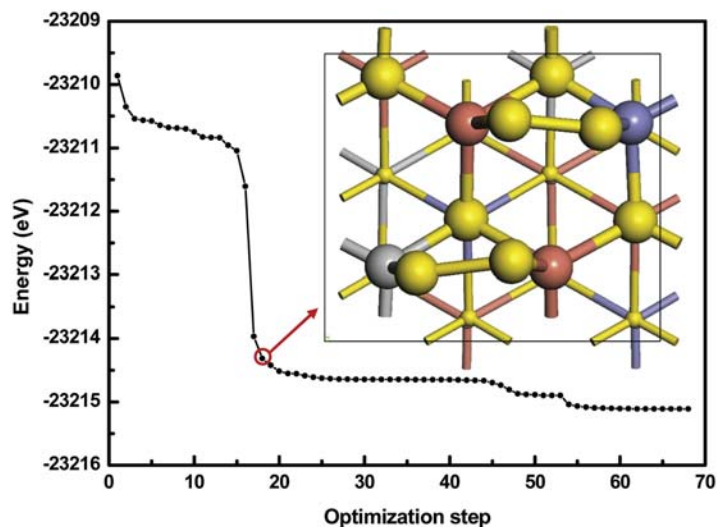


FIGURE 2.17 Total energy changes with geometry optimization of (112)-S chalcopyrite surface. The inset shows the geometry of the 18th step of optimization.

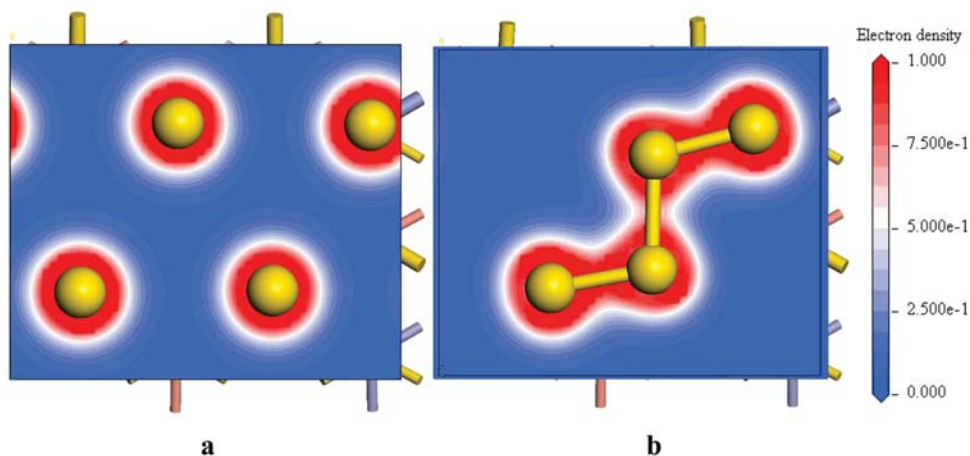


FIGURE 2.18 Electron density of (A) unreconstructed and (B) reconstructed (001)-S chalcopyrite surface. Electron density of (C) unreconstructed and (D) reconstructed (112)-S chalcopyrite surface.

S—S bond formed in an oxidative process. Fig. 2.19B shows that the number of occupied electronic states on Fe atoms in the region of -1 to 0 eV increased owing to the reconstruction, but similar behavior was not observed for the LDOS on Cu atoms (Fig. 2.19C). Similar to reconstruction of the (001)-S terminated surface, electrons released with the formation of S_4^{2-} were transferred to Fe atoms.

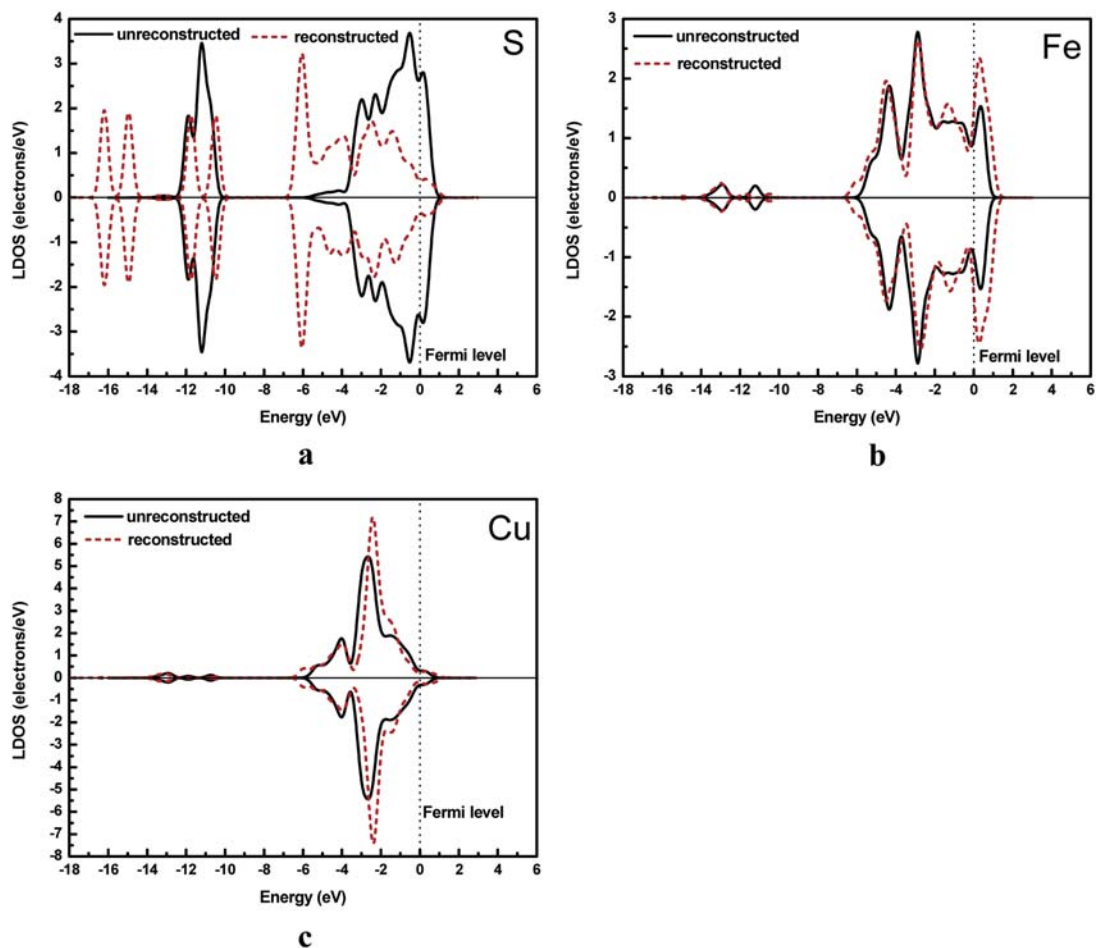


FIGURE 2.19 Density of states on (A) sulfur atoms, (B) iron atoms, and (C) copper atoms in reconstructed (112)-S chalcopyrite surface. *LDOS*, local density of states.

2.4 Summary

The Cu 2p spectrum of CuO and $\text{Cu}_2(\text{OH})_2\text{CO}_3$ has a strong satellite peak near 942 eV, whereas the Cu 2p spectrum of Cu_2O has only a weak satellite near 945 eV, and no obvious satellite peaks are observed in the Cu 2p spectra of Cu_2S . The Cu 2p spectrum of CuFeS_2 is almost the same for Cu_2O and Cu_2S . Thus, the oxidation state for copper in chalcopyrite is expected to be Cu(I). The DFT calculation results show that Cu atoms and Fe atoms transfer 0.17 and 0.47 electrons to S atoms, respectively, and the electron transfer ratios of Cu and Fe are close to 1:3. This further proves that the oxidation states of Cu, Fe, and S in chalcopyrite are +1, +3, and -2, respectively. Therefore, chalcopyrite can be written as $\text{Cu}^{\text{I}}\text{Fe}^{\text{III}}\text{S}_2$.

S_2^{2-} and S_n^{2-} were detected on the surface of chalcopyrite after polishing and exposure to air for no more than a few minutes. DFT calculation results also indicated that the chalcopyrite (001)-S and (112)-S surface undergoes important reconstruction forming S_2^{2-} and S_4^{2-} , respectively; and S_2^{2-} is the precursor to forming S_4^{2-} . Electrons released with the formation of S_2^{2-} or S_4^{2-} are transferred to Fe atoms, causing an increase in the number of states on Fe atoms near the Fermi level (−1 to 0 eV).

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No. 51904339), National Basic Research Program of China (Grant No. 2010CB630905), High-Tech Research and Development Program of China (Grant No. 2012AA061501). I also thank Prof. Wenqing Qin for his valuable advice.

References

- Acres, R. G., Harmer, S. L., & Beattie, D. A. (2010a). Synchrotron XPS, NEXAFS, and ToF-SIMS studies of solution exposed chalcopyrite and heterogeneous chalcopyrite with pyrite. *Minerals Engineering*, 23(11–13), 928–936. <https://doi.org/10.1016/j.mineng.2010.03.007>.
- Acres, R. G., Harmer, S. L., & Beattie, D. A. (2010b). Synchrotron XPS studies of solution exposed chalcopyrite, bornite, and heterogeneous chalcopyrite with bornite. *International Journal of Mineral Processing*, 94(1–2), 43–51. <https://doi.org/10.1016/j.minpro.2009.11.006>.
- Boekema, C., Krupski, A. M., Varasteh, M., Parvin, K., Van Til, F., Van Der Woude, F., et al. (2004). Cu and Fe valence states in $CuFeS_2$. *Journal of Magnetism and Magnetic Materials*, 272–276(Issue I), 559–561. <https://doi.org/10.1016/j.jmmm.2003.11.206>. Elsevier.
- Burdick, C. L., & Ellis, J. H. (1917). The crystal structure of chalcopyrite determined by X-rays. *Journal of the American Chemical Society*, 39(12), 2518–2525. <https://doi.org/10.1021/ja02257a002>.
- Chen, J., Long, X., & Chen, Y. (2014). Comparison of multilayer water adsorption on the hydrophobic galena (PbS) and hydrophilic pyrite (FeS₂) surfaces: A DFT study. *Journal of Physical Chemistry C*, 118(22), 11657–11665. <https://doi.org/10.1021/jp5000478>.
- Chusuei, C. C., Brookshier, M. A., & Goodman, D. W. (1999). Correlation of relative X-ray photoelectron spectroscopy shake-up intensity with CuO particle size. *Langmuir*, 15(8), 2806–2808. <https://doi.org/10.1021/la9815446>.
- De Lima, G. F., De Oliveira, C., De Abreu, H. A., & Duarte, H. A. (2011). Water adsorption on the reconstructed (001) chalcopyrite surfaces. *Journal of Physical Chemistry C*, 115(21), 10709–10717. <https://doi.org/10.1021/jp201106e>.
- De Oliveira, C., De Lima, G. F., De Abreu, H. A., & Duarte, H. A. (2012). Reconstruction of the chalcopyrite surfaces-A DFT study. *Journal of Physical Chemistry C*, 116(10), 6357–6366. <https://doi.org/10.1021/jp300713z>.
- De Oliveira, C., & Duarte, H. A. (2010). Disulphide and metal sulphide formation on the reconstructed (0 0 1) surface of chalcopyrite: A DFT study. *Applied Surface Science*, 257(4), 1319–1324. <https://doi.org/10.1016/j.apsusc.2010.08.059>.
- Edelbro, R., Sandström, A., & Paul, J. (2003). Full potential calculations on the electron bandstructures of Sphalerite, Pyrite and Chalcopyrite. *Applied Surface Science*, 206(1–4), 300–313. [https://doi.org/10.1016/S0169-4332\(02\)01284-9](https://doi.org/10.1016/S0169-4332(02)01284-9).
- Gahremaninezhad, A., Dixon, D. G., & Asselin, E. (2013). Electrochemical and XPS analysis of chalcopyrite ($CuFeS_2$) dissolution in sulfuric acid solution. *Electrochimica Acta*, 87, 97–112. <https://doi.org/10.1016/j.electacta.2012.07.119>.
- Goh, S. W., Buckley, A. N., & Lamb, R. N. (2006). Copper(II) sulfide? *Minerals Engineering*, 19(2), 204–208. <https://doi.org/10.1016/j.mineng.2005.09.003>.
- Goh, S. W., Buckley, A. N., Lamb, R. N., Rosenberg, R. A., & Moran, D. (2006). The oxidation states of copper and iron in mineral sulfides, and the oxides formed on initial exposure of chalcopyrite and bornite to air. *Geochimica et Cosmochimica Acta*, 70(9), 2210–2228. <https://doi.org/10.1016/j.gca.2006.02.007>.
- Hall, S. R., & Stewart, J. M. (1973). The crystal structure refinement of chalcopyrite, $CuFeS_2$. *Acta Crystallographica Section B Structural Crystallography and Crystal Chemistry*, 579–585. <https://doi.org/10.1107/S0567740873002943>.

- Harmer, S. L., Pratt, A. R., Nesbitt, H. W., & Fleet, M. E. (2005). Reconstruction of fracture surfaces on bornite. *The Canadian Mineralogist*, 43(5), 1619–1630. <https://doi.org/10.2113/gscanmin.43.5.1619>.
- Himmetoglu, B., Floris, A., De Gironcoli, S., & Cococcioni, M. (2014). Hubbard-corrected DFT energy functionals: The LDA+U description of correlated systems. *International Journal of Quantum Chemistry*, 114(1), 14–49. <https://doi.org/10.1002/qua.24521>.
- Klauber, C. (2003). Fracture-induced reconstruction of a chalcopyrite (CuFeS₂) surface. *Surface and Interface Analysis*, 35(5), 415–428. <https://doi.org/10.1002/sia.1539>.
- Kundu, M., Hasegawa, T., Terabe, K., & Aono, M. (2008). Effect of sulfurization conditions on structural and electrical properties of copper sulfide films. *Journal of Applied Physics*, 103(7). <https://doi.org/10.1063/1.2903599>.
- Kundu, M., Hasegawa, T., Terabe, K., Yamamoto, K., & Aono, M. (2008). Structural studies of copper sulfide films: Effect of ambient atmosphere. *Science and Technology of Advanced Materials*, 9(3). <https://doi.org/10.1088/1468-6996/9/3/035011>.
- van der Laan, G., Patrick, R. A. D., Henderson, C. M. B., & Vaughan, D. J. (1992). Oxidation state variations in copper minerals studied with Cu 2p X-ray absorption spectroscopy. *Journal of Physics and Chemistry of Solids*, 53(9), 1185–1190. [https://doi.org/10.1016/0022-3697\(92\)90037-E](https://doi.org/10.1016/0022-3697(92)90037-E).
- Li, Y., Chandra, A. P., & Gerson, A. R. (2014). Scanning photoelectron microscopy studies of freshly fractured chalcopyrite exposed to O₂ and H₂O. *Geochimica et Cosmochimica Acta*, 133, 372–386. <https://doi.org/10.1016/j.gca.2014.02.037>.
- Li, Y., Kawashima, N., Li, J., Chandra, A. P., & Gerson, A. R. (2013). A review of the structure, and fundamental mechanisms and kinetics of the leaching of chalcopyrite. *Advances in Colloid and Interface Science*, 197–198, 1–32. <https://doi.org/10.1016/j.cis.2013.03.004>.
- Mikhlin, Y. L., Tomashevich, Y. V., Asanov, I. P., Okotrub, A. V., Varnek, V. A., & Vyalikh, D. V. (2004). Spectroscopic and electrochemical characterization of the surface layers of chalcopyrite (CuFeS₂) reacted in acidic solutions. *Applied Surface Science*, 225(1–4), 395–409. <https://doi.org/10.1016/j.apsusc.2003.10.030>.
- Mikhlin, Tomashevich, Y., Tauson, V., Vyalikh, D., Molodtsov, S., & Szargan, R. (2005). A comparative X-ray absorption near-edge structure study of bornite, Cu₅FeS₄, and chalcopyrite, CuFeS₂. *Journal of Electron Spectroscopy and Related Phenomena*, 142(1), 83–88. <https://doi.org/10.1016/j.elspec.2004.09.003>.
- Mishra, K. K., & Osseo-Asare, K. (1988). Aspects of the interfacial electrochemistry of semiconductor pyrite (FeS₂). *Journal of the Electrochemical Society*, 135(10), 2502–2509. <https://doi.org/10.1149/1.2095366>.
- Nava, D., González, I., Leinen, D., & Ramos-Barrado, J. R. (2008). Surface characterization by X-ray photoelectron spectroscopy and cyclic voltammetry of products formed during the potentiostatic reduction of chalcopyrite. *Electrochimica Acta*, 53(14), 4889–4899. <https://doi.org/10.1016/j.electacta.2008.01.088>.
- Rais, A., Gismelseed, A. M., & Al-Rawas, A. D. (2000). Magnetic properties of natural chalcopyrite at low temperature. *Materials Letters*, 46(6), 349–353. [https://doi.org/10.1016/S0167-577X\(00\)00199-3](https://doi.org/10.1016/S0167-577X(00)00199-3).
- Stirling, A., Bernasconi, M., & Parrinello, M. (2003). Ab initio simulation of water interaction with the (100) surface of pyrite. *The Journal of Chemical Physics*, 118(19), 8917–8926. <https://doi.org/10.1063/1.1566936>.
- Szpunar, B. (2012). Investigation of urania within LDAU method. *Journal of Physics and Chemistry of Solids*, 73(8), 1003–1009. <https://doi.org/10.1016/j.jpccs.2012.03.007>.
- Todd, E. C., Sherman, D. M., & Purton, J. A. (2003). Surface oxidation of chalcopyrite (CuFeS₂) under ambient atmospheric and aqueous (pH 2–10) conditions: Cu, Fe L- and O K-edge X-ray spectroscopy. *Geochimica et Cosmochimica Acta*, 67(12), 2137–2146. [https://doi.org/10.1016/S0016-7037\(02\)01371-6](https://doi.org/10.1016/S0016-7037(02)01371-6).
- Ueda, H., Nohara, M., Kitazawa, K., Takagi, H., Fujimori, A., Mizokawa, T., et al. (2002). Copper pyrites CuS₂ and CuSe₂ as anion conductors. *Physical Review B: Condensed Matter and Materials Physics*, 65(15), 1551041–1551045.
- Yin, M., Wu, C. K., Lou, Y., Burda, C., Koberstein, J. T., Zhu, Y., et al. (2005). Copper oxide nanocrystals. *Journal of the American Chemical Society*, 127(26), 9506–9511. <https://doi.org/10.1021/ja050006u>.

This page intentionally left blank

Electrochemical dissolution process of chalcopyrite

3.1 Electrochemical behavior of chalcopyrite

Generally, different in situ electrochemical techniques (e.g., cyclic voltammetry, potentiodynamic and potentiostatic polarization, electrochemical impedance spectroscopy (EIS), and chronoamperometry) and ex situ spectroscopic methods (e.g., X-ray photoelectron spectroscopy, synchrotron small angle X-ray diffraction (XRD), and Raman spectroscopy) have been applied to understand the dissolution mechanism of chalcopyrite and characterize the surface layers on electrodes (Ghahremaninezhad, Dixon, & Asselin, 2012, 2013; Majuste, Ciminelli, Osseo-Asare, Dantas, & Magalhães-Paniago, 2012; Nava & González, 2006). However, uncertainty remains regarding the composition of the passive film that forms during leaching.

Over the past decades, several researchers have investigated the passivation film formed in the chalcopyrite dissolution process. Disulfide (S_2^{2-}) (Acres, Harmer, & Beattie, 2010b; Klauber, 2008; Klauber, Parker, Van Bronswijk, & Watling, 2001; Parker, Klauber, Kougianos, Watling, & Van Bronswijk, 2003), polysulfide (S_n^{2-}) (Acres, Harmer, & Beattie, 2010b; Ghahremaninezhad, Dixon, & Asselin, 2013; Wu et al., 2015), metal-deficient sulfide ($Cu_{1-x}Fe_{1-y}S_{2-z}$) ($y > x$) (Ghahremaninezhad, Dixon, & Asselin, 2013; Majuste, Ciminelli, Osseo-Asare, Dantas, & Magalhães-Paniago, 2012; Nava & González, 2006), CuS_2 (Yin et al., 1995), CuS (Majuste, Ciminelli, Osseo-Asare, Dantas, & Magalhães-Paniago, 2012; Zhao et al., 2015), Cu_5FeS_4 (Majuste et al., 2012), elemental sulfur (S^0) (Klauber, 2008; Klauber, Parker, Van Bronswijk, & Watling, 2001; Sasaki, Takatsugi, & Tuovinen, 2012), and jarosites ($MFe_3[SO_4]_2[OH]_6$, where M is a monovalent cation, e.g., K^+ , Na^+ , NH_4^+ , and H_3O^+) (Harmer, Thomas, Fornasiero, & Gerson, 2006; He et al., 2009; Kinnunen, Heimala, Riekkola-Vanhanen, & Puhakka, 2006; Klauber, 2008; Parker, Klauber, Kougianos, Watling, & Van Bronswijk, 2003; Sandström, Shchukarev, & Paul, 2005; Xia et al., 2010; Zhu et al., 2011) have been proposed as passivation candidates. Nava and González (2006) found that the initial chalcopyrite dissolution occurred at $0.615 \text{ V} \leq E_{anod} < 1.015 \text{ V}$ and formed $Cu_{1-x}Fe_{1-y}S_{2-z}$ that passivated the electrode surface. Majuste et al. (2012) stated that $Cu_{1-x}Fe_{1-y}S_{2-z}$, Cu_5FeS_4 and CuS hinder chalcopyrite dissolution, but S^0 seemed to have no impact on the electrochemical dissolution of chalcopyrite. Yin et al. (1995) reported that CuS_2 passivates chalcopyrite surfaces at lower potentials

and S^0 passivates chalcopyrite surfaces at higher potentials in a strongly acidic electrolyte. S_3^{2-} and S_4^{2-} were identified by Mikhlin et al. (2004) on the surface of chalcopyrite after leaching with $H_2SO_4-Fe_2(SO_4)_3$ and $HCl-FeCl_3$, respectively. S_2^{2-} and S_n^{2-} were reported by Acres et al. (2010b) and Harmer et al. (2006) on the surfaces of chalcopyrite in chemical leaching; both were also detected by Zhao et al. (2015) during the bioleaching of chalcopyrite. Klauber et al. (2001) interpreted, S^0 and S_2^{2-} as two main passivation candidates for ferric leaching inhibition. He et al. (2009) and Zhu et al. (2011) stated that jarosite was the major passivation composition hindering the bioleaching of chalcopyrite by thermophilic archaea, whereas S^0 seemed to have no impact on the bioleaching of chalcopyrite. D'Hugues et al. (2002) and Gu et al. (2013) found no evidence of jarosite hindrance to chalcopyrite chemical leaching or bioleaching. Qin et al. (2013) reported that 92.5% Cu was extracted from chalcopyrite by moderately thermophilic microorganisms, even though a significant amount of S^0 and jarosite formed on the surface of chalcopyrite; similar results were obtained by Yang et al. (2015).

Chalcopyrite passive film was studied by applying cyclic voltammetry (CV), potentiodynamic, potentiostatic, and Tafel polarization in pH 1.6 electrolyte solutions at 50°C. The products formed during electrochemical treatment of chalcopyrite were analyzed by X-ray photoelectron spectroscopy (XPS). All electrochemical experiments were conducted with a Princeton Model 283 potentiostat (EG&G of Princeton Applied Research) controlled by PowerSuite under ambient atmosphere at 50°C in an electrolyte solution. A conventional three-electrode electrolytic cell with a graphite rod as a counter electrode, a saturated Ag/AgCl electrode as a reference electrode, and the chalcopyrite electrode with 1-cm² areas exposed to the solution as the working electrode were used for electrochemical analysis. The electrolyte solution was prepared as follows: 3.0 g/L $(NH_4)_2SO_4$, 0.1 g/L KCl, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L K_2HPO_4 , 0.01 g/L $Ca(NO_3)_2$ with a pH of 1.6. Before every electrochemical experiment, the chalcopyrite electrode surface was polished with carbide papers from numbers 800 to 3000, and then rinsed with deionized water. Potentiodynamic polarization was scanned from 200 to 1000 mV at a scan rate of 20 mV/s. Potentiostatic polarization was conducted at 475, 600, 700, 800, 900, and 1000 mV for 1800 s, respectively. The cyclic voltammograms and Tafel polarization curves were obtained after electrochemical treatment of a chalcopyrite electrode at 475, 600, 700, 800, 900, and 1000 mV for 1800 s. CV was scanned from 475 mV in the positive direction at a scan rate of 20 mV/s. The scan rate of the Tafel polarization is 0.5 mV/s. Corrosion current density (I_{corr}) was obtained with extension of the cathodic Tafel slope to the corrosion potential (E_{corr}), and polarization resistance (R_p) was obtained with fitting of the Tafel polarization curves at $E_{corr} \pm 20$ mV.

The E-t curve of chalcopyrite in an electrolyte solution is shown Fig. 3.1. When chalcopyrite electrode is immersed into electrolyte, the potential of the electrode increases rapidly and then gradually reaches a stable state. The potential of the chalcopyrite electrode finally stabilized at 475 mV after 1800 s, which is an open circuit potential (OCP).

The cyclic voltammogram of massive chalcopyrite electrodes is shown in Fig. 3.2A. The potential of the electrode was initially swept from the OCP (475 mV) in a positive direction with a scan rate of 20 mV/s. Five anodic peaks (A1, A2, A3, A4, and A5) and four cathodic peaks (C1, C2, C3, and C4) were detected.

Chalcopyrite begins to dissolve when it is scanned from the OCP to 600 mV (A1). According to the literature, iron preferentially dissolves from the crystal lattice of chalcopyrite and forms metal-deficient sulfide ($Cu_{1-x}Fe_{1-y}S_{2-z}$) ($y > x$) (Eq. 3.1) (Eghbalian & Dixon, 2011;

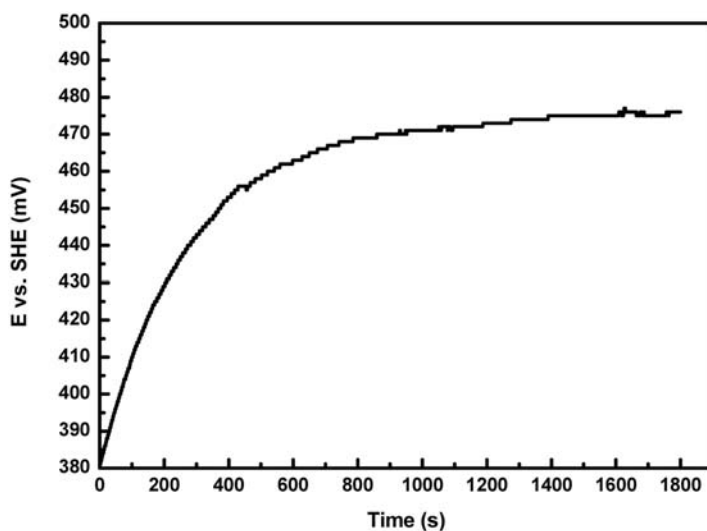


FIGURE 3.1 Open circuit potential of massive chalcopyrite electrodes in the medium. From Yang, C., Jiao, F., & Qin, W. (2018). Co-bioleaching of chalcopyrite and silver-bearing bornite in a mixed moderately thermophilic culture. *Minerals*, 8(1), 4. <https://doi.org/10.3390/min8010004>.

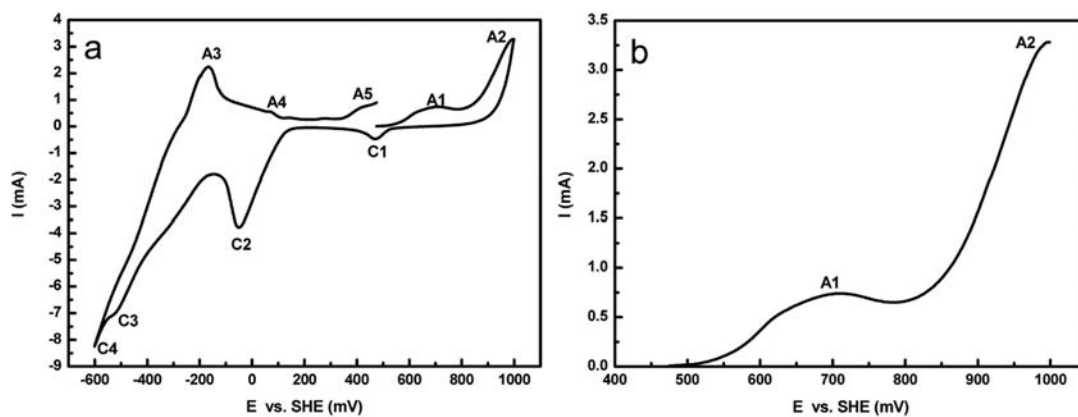
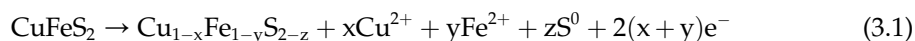
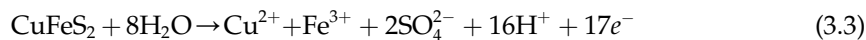
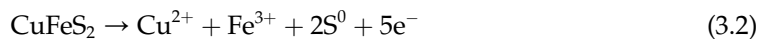


FIGURE 3.2 Cyclic voltammograms (A) and cyclic voltammogram focusing on peak A₁ and A₂ (B) of chalcopyrite electrode. From Wu, S., Yang, C., Qin, W., Jiao, F., Wang, J., & Zhang, Y. (2015). Sulfur composition on surface of chalcopyrite during its bioleaching at 50°C. *Transactions of Nonferrous Metals Society of China*, 25(12), 4110–4118. [https://doi.org/10.1016/S1003-6326\(15\)64062-6](https://doi.org/10.1016/S1003-6326(15)64062-6).

Elsherief, 2002; Liang et al., 2011; Mikhlin et al., 2004). When the scan continues to move near 1000 mV (A₂), the chalcopyrite is completely dissolved into copper and iron ions (Eqs. 3.2–3.3) (Gómez, Figueroa, Muñoz, Blázquez, & Ballester, 1996; Liang et al., 2011; López-Juárez, Gutiérrez-Arenas, & Rivera-Santillán, 2006):

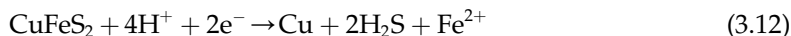
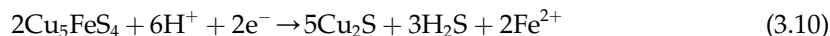
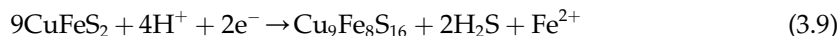
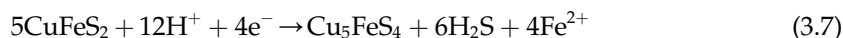




When the sweeping potential is switched to a negative direction at 1000 mV, a cathodic peak C1 is detected at about 500 mV. Peak C1 should represent the reduction of ferric ions according to Eq. (3.4) (Eghbalnia & Dixon, 2011; Gómez, Figueroa, Muñoz, Blázquez, & Bal-lester, 1996; Zeng, Qiu, Zhou, & Chen, 2011). Alternatively, this peak can be attributed to the reduction of copper ions and elemental sulfur to covellite (Eq. 3.5) and/or the reduction of copper ions and covellite to chalcocite (Eq. 3.6) (Gómez, Figueroa, Muñoz, Blázquez, & Bal-lester, 1996; Lee, Nicol, & Basson, 2008; Liang et al., 2011; López-Juárez, Gutiérrez-Arenas, & Rivera-Santillán, 2006; Mikhlin et al., 2004):



In the potential range of 100 to −100 mV, peak C2 should represent the initial reduction of chalcopyrite, as shown in Eqs. (3.7)–(3.8) (Eghbalnia & Dixon, 2011; Liang et al., 2011; Mikhlin et al., 2004). Nava et al. (2008) and Eghbalnia and Dixon (2011) assigned this cathodic peak, which appeared in the potential domain of 115 to −85 mV, to chalcopyrite reduction to talnakhite-type compounds, as shown in Eq. (3.9). When the scan continued moving in the negative direction, the reduction of bornite and chalcopyrite to chalcocite took place in the potential range of −100 to −560 mV (Eqs. 3.10–3.11) (Eghbalnia & Dixon, 2011; Liang et al., 2011; Mikhlin et al., 2004). When the scanning potential is below −560 mV, the chalcopyrite and/or chalcocite is reduced to metal copper (Eqs. 3.12–3.13):



When the sweeping potential was switched to a positive direction at -600 mV, an anodic peak (A3) was detected near -200 mV. This peak can be attributed to the reduction of metal copper and hydrogen sulfide to chalcocite (Eq. 3.14) (Gu, Hu, Zhang, Xiong, & Yang, 2013; Liang et al., 2011). Peak A4 (~ 50 mV) might represent the oxidation of hydrogen sulfide to elemental sulfur, as illustrated in Eq. (3.15) (Eghbalian & Dixon, 2011; Elsherief, 2002; Liang et al., 2011). Chalcocite is oxidized to a series of nonstoichiometric sulfides, Cu_{2-x}S , when the potential is above 400 mV (Eq. 3.16) (Eghbalian & Dixon, 2011; Elsherief, 2002; Liang et al., 2011; Mikhlin et al., 2004; Nava & González, 2006; Nava, González, Leinen, & Ramos-Barrado, 2008; Zeng, Qiu, Zhou, & Chen, 2011):

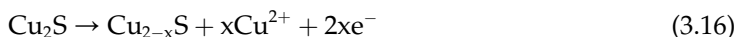
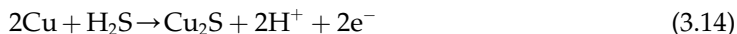


Fig. 3.2B shows that the anodic oxidation current decreased (from 0.74 to 0.66 mA) with an increase in the sweeping potential from 710 to 800 mV, which indicated that the product of chalcopyrite dissolution in the potential range of 520 – 710 mV accumulated on the surface and passivated the electrode surface.

To find out whether the oxidation product of peak A1 or A2 caused the passivation of the electrode surface, multicycle voltammetry with different potential intervals was carried out. Fig. 3.3 shows the multicyclic voltammogram (10 cycles) of the chalcopyrite electrode in the potential window of 475 – 1000 mV. In this experiment, the potential of the electrode was initially swept from 475 mV to 600 , 700 , 800 , 900 , and 1000 mV, and the following nine cycles were swept from 550 mV.

Fig. 3.3A shows a multicyclic voltammogram of the chalcopyrite electrode in the potential window of 475 – 600 mV. In the first cycle, the anodic oxidation current increases with an increase of the sweeping potential, but the peak current is only 138 μA . The low anodic oxidation current indicates that the chalcopyrite is only slightly oxidized at this potential interval. As the number of scans increases, the peak current gradually decreases and the peak current is reduced from 138 to 45.5 μA after 10 cycles. The decrease in the peak current of anodic oxidation indicates that the oxidation product accumulates on the surface of the electrode and passivates the chalcopyrite.

Fig. 3.3B shows a multicyclic voltammogram of the chalcopyrite electrode in the potential window of 475 – 700 mV. In the first cycle, the anodic oxidation current increases with an increase of the sweeping potential and reaches 298 μA . However, the anodic oxidation current decreases to 134.6 μA in the second cycle; the peak current is finally reduced from 298 to 42.4 μA after 10 cycles. Therefore, the oxidation product formed in the range of 475 – 700 mV accumulates on the surface of the electrode and passivates the chalcopyrite.

Fig. 3.3C shows the multicyclic voltammogram of the chalcopyrite electrode in the potential window of 475 – 800 mV. In the first cycle, the anodic oxidation current increases with an increase in potential from 520 to 700 mV and reaches 360 μA . However, the slope of cyclic voltammogram is reduced when the sweeping potential increases from 700 to 740 mV,

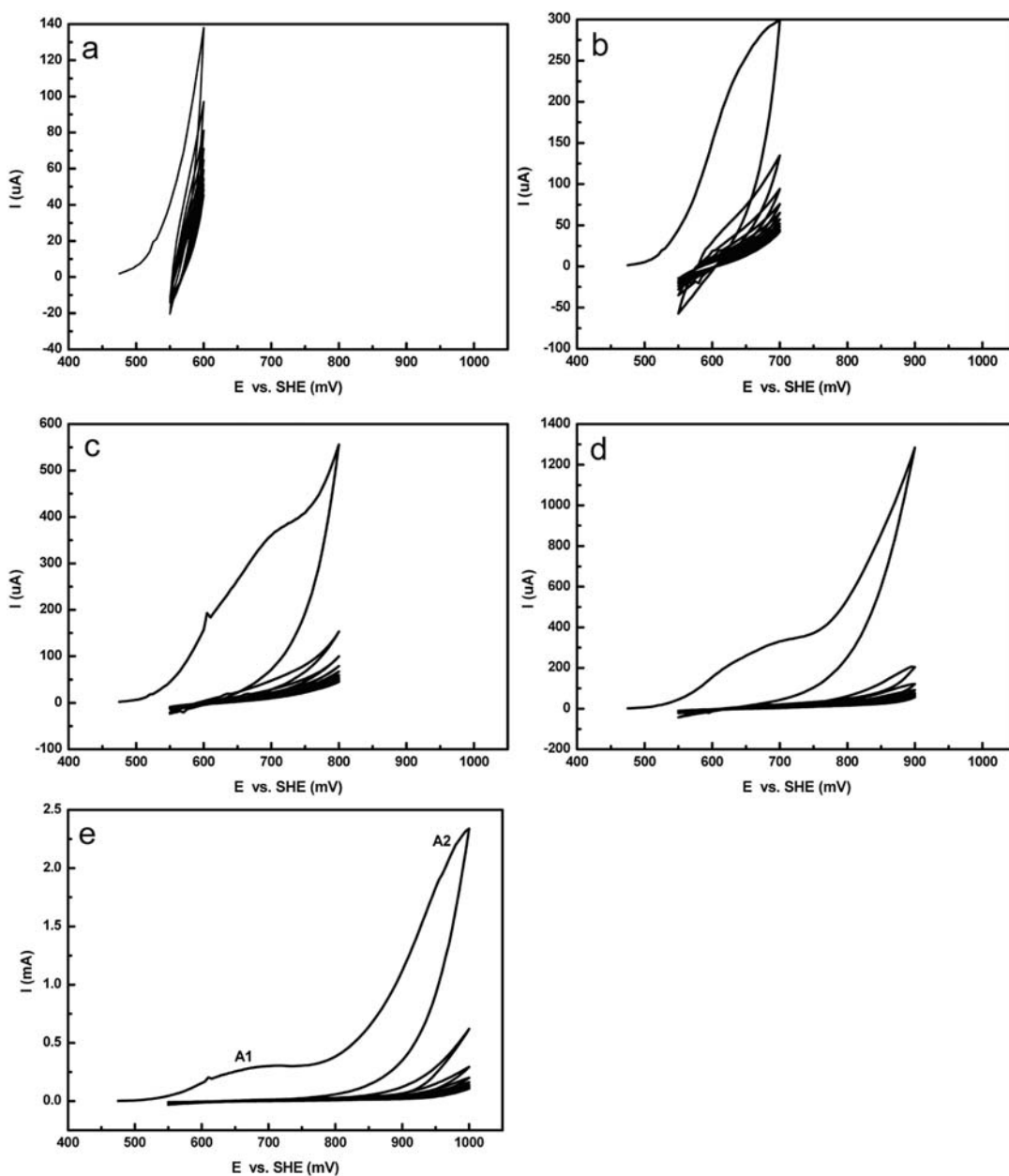


FIGURE 3.3 Multicyclic voltammogram (10 cycles, SR = 20 mV/s) of chalcopyrite electrode: (A) 475–600 mV, (B) 475–700 mV, (C) 475–800 mV, (D) 475–900 mV, and (E) 475–1000 mV. From Wu, S., Yang, C., Qin, W., Jiao, F., Wang, J., & Zhang, Y. (2015). Sulfur composition on surface of chalcopyrite during its bioleaching at 50°C. *Transactions of Nonferrous Metals Society of China* 25(12), 4110–4118. [https://doi.org/10.1016/S1003-6326\(15\)64062-6](https://doi.org/10.1016/S1003-6326(15)64062-6).

indicating that the current increase rate decreases in this potential range. In other words, the oxidation product formed in the range of 520–700 mV accumulates on the surface of the electrode and passivates the chalcopyrite. The slope of the cyclic voltammogram increases when the sweeping potential increases from 740 to 800 mV, indicating increase of current owing to the dissolution of passivation film. The peak current finally reaches 556 μA . The anodic oxidation current decreases to 153.2 μA in the second cycle; the peak current is finally reduced to 45.5 μA after 10 cycles. The multicyclic voltammograms of the chalcopyrite electrode in the potential window of 475–900 mV (Fig. 3.3D) and 475–1000 mV (Fig. 3.3E) are similar to that in the potential window of 475–800 mV. Therefore, the oxidation product formed in the range of 475–700 mV accumulates on the surface of the electrode and passivates the chalcopyrite; the dissolution of passivation film occurs when the sweeping potential is above 740 mV, but the products of passivation film oxidation will also passivate the electrode surface.

Fig. 3.4 shows the potentiodynamic polarization of chalcopyrite. The initial oxidation of chalcopyrite occurs in region A (potentials between OCP and 600 mV), in which the small quantity of chalcopyrite was oxidized. The change in slope in region B (potentials between 600 and 700 mV) illustrates that the product of chalcopyrite dissolution in the potential window of the OCP to 600 mV accumulated on the surface and passivated the electrode surface. The electrode shows significant dissolution in regions C (potentials between 700 and 830 mV) and E (above 920 mV). In region D (potentials between 830 and 920 mV), the chalcopyrite surface appears to be in the passive state.

Fig. 3.5 presents the i – t behavior of chalcopyrite for applied potentials at 475, 600, 700, 800, 900, and 1000 mV. The i – t curves of chalcopyrite for applied potentials in the potential

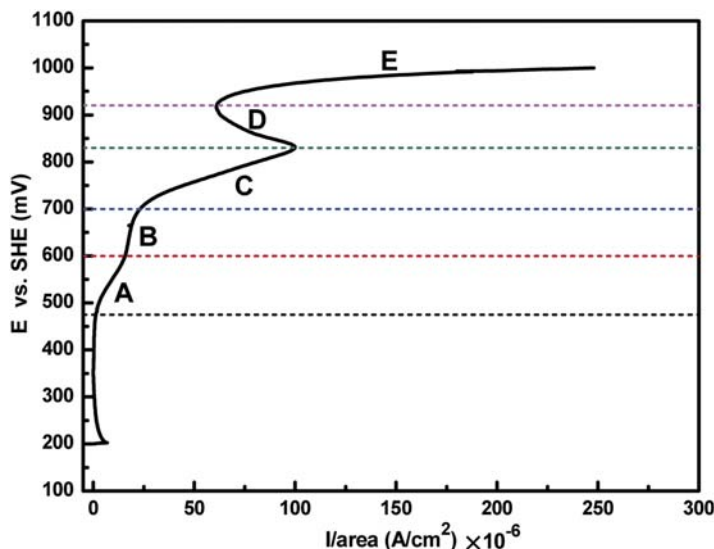


FIGURE 3.4 Potentiodynamic polarization of chalcopyrite electrode. The scan rate is 0.5 mV/s. From Yang, C., Qin, W., Zhao, H., Wang, J., & Wang, X. (2018). Mixed potential plays a key role in leaching of chalcopyrite: Experimental and theoretical analysis. *Industrial and Engineering Chemistry Research*, 57(5), 1733–1744. <https://doi.org/10.1021/acs.iecr.7b02051>.

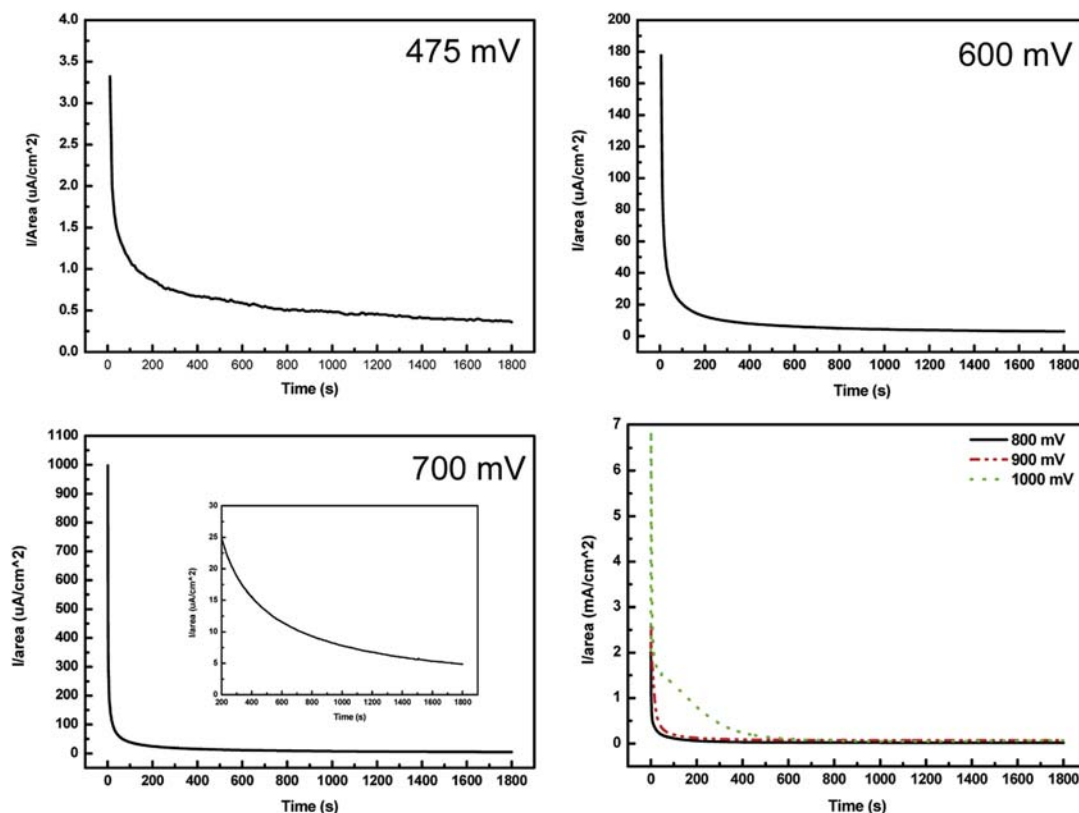


FIGURE 3.5 Potentiostatic polarization of chalcopyrite at 475, 600, 700, 800, 900, and 1000 mV for 30 min. Inset figures show an expanded view of potentiostatic polarization of chalcopyrite at 700 mV. From Yang, C., Qin, W., Zhao, H., Wang, J., & Wang, X. (2018). Mixed potential plays a key role in leaching of chalcopyrite: Experimental and theoretical analysis. *Industrial and Engineering Chemistry Research*, 57(5), 1733–1744. <https://doi.org/10.1021/acs.iecr.7b02051>.

window of 475–900 mV show that the current density decreases sharply owing to the formation of a stable passive film during the first 300 s. However, potentiostatic polarization of chalcopyrite at 1000 mV shows that the current density decreases sharply during the first 50 s, and then the current density slowly decreases owing to accumulation of the product of oxidized chalcopyrite on the surface. The electrode surface reaches a steady-state current after 600 s. The passive layer formed on the chalcopyrite surface can be further confirmed using cyclic voltammograms obtained after the dissolution of chalcopyrite at 475, 600, 700, 800, 900, and 1000 mV for 1800 s (Fig. 3.6). Fig. 3.6 shows that the passive film formed in the potential window of 475–700 mV would be oxidized when the sweeping potential is above 800 mV.

Tafel polarization curves obtained after electrochemical treatment of a chalcopyrite electrode at 475, 600, 700, 800, 900, and 1000 mV for 1800 s are shown in Fig. 3.7A. From the Tafel polarization curves, E_{corr} , I_{corr} , and R_p can be obtained (as presented in Fig. 3.7B–D). Increasing the polarization potential leads to a significant increase in the E_{corr} (Fig. 3.7B). The R_p increase with an increase in polarization potential from 475 to 700 mV suggests the

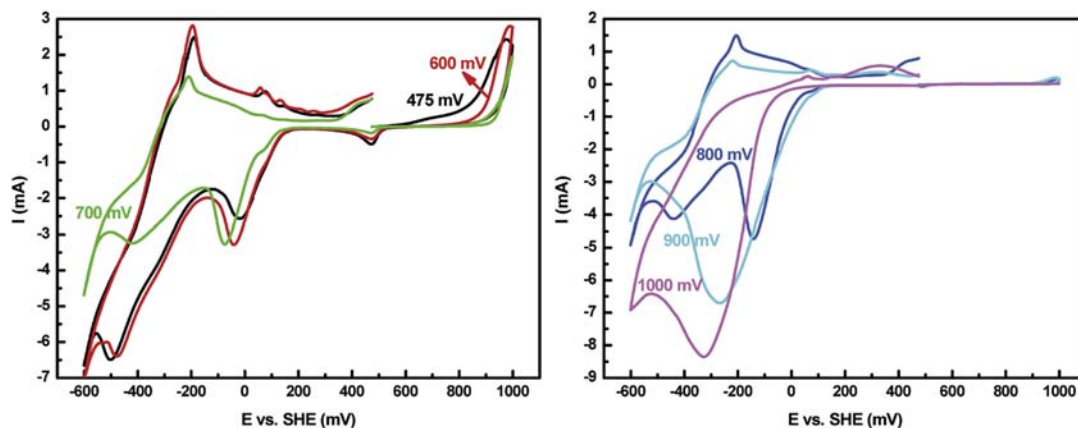


FIGURE 3.6 Cyclic voltammograms of chalcopyrite after dissolution at 475, 600, 700, 800, 900, and 1000 mV for 30 min. From Yang, C., Qin, W., Zhao, H., Wang, J., & Wang, X. (2018). Mixed potential plays a key role in leaching of chalcopyrite: Experimental and theoretical analysis. *Industrial and Engineering Chemistry Research*, 57(5), 1733–1744. <https://doi.org/10.1021/acs.iecr.7b02051>.

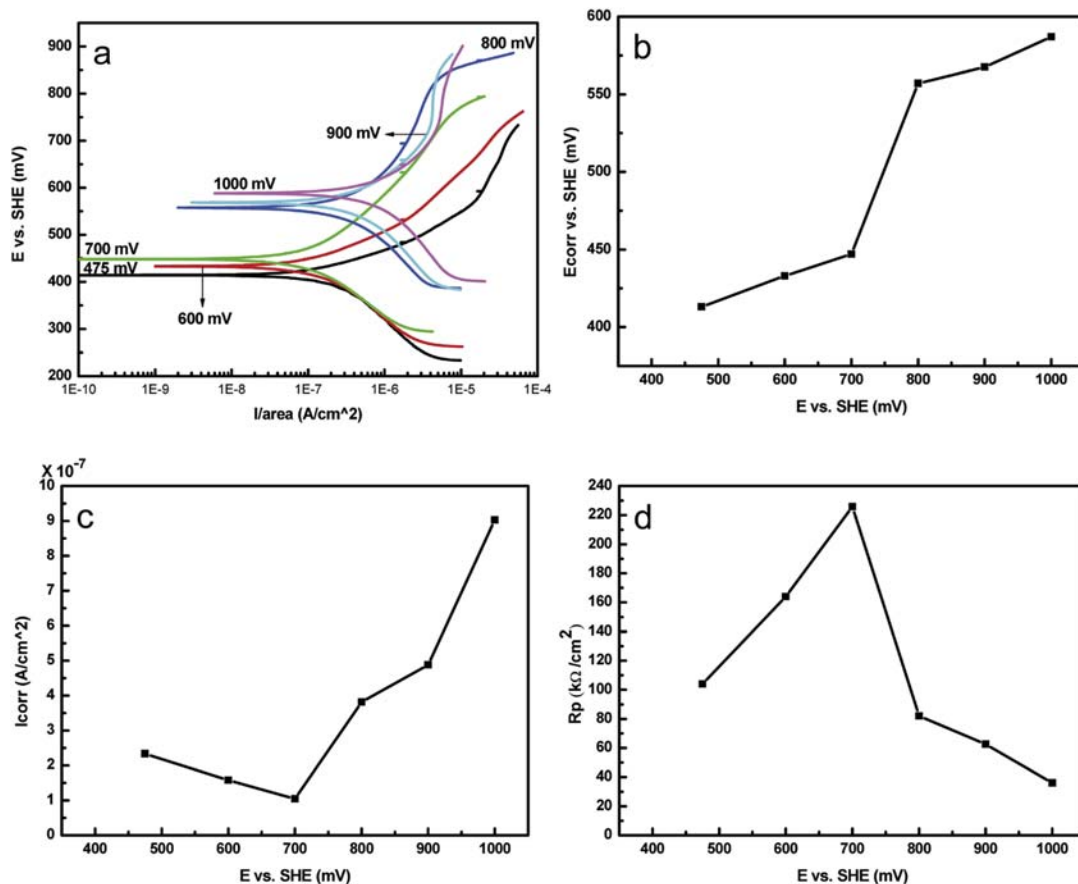


FIGURE 3.7 Tafel plot for chalcopyrite electrode (A) and obtained electrochemical corrosion kinetic parameters: (B) corrosion potential, (C) corrosion current density, and (D) polarization resistance. The scan rate is 0.5 mV/s. From Yang, C., Qin, W., Zhao, H., Wang, J., & Wang, X. (2018). Mixed potential plays a key role in leaching of chalcopyrite: Experimental and theoretical analysis. *Industrial and Engineering Chemistry Research*, 57(5), 1733–1744. <https://doi.org/10.1021/acs.iecr.7b02051>.

formation of passivation films on the chalcopyrite surface. In general, a passivation layer on the material surface hinders the dissolution owing to its low electron or ionic conduction. Ghahremaninezhad et al. (2012) reported that the passive film hinders the dissolution of chalcopyrite because of its low ionic conduction. When the polarization potential increased from 700 to 1000 mV, the accumulation of product on the surface of the chalcopyrite resulted in decreases in the current (Fig. 3.5), but the R_p decreased with an increase in the polarization potential, which suggests that chalcopyrite transpassive dissolution occurs. Fig. 3.7C shows that a decrease in current density from 0.23 to 0.10 $\mu\text{A}/\text{cm}^2$ with polarization potential increased from 475 to 700 mV, and then the current density increased from 0.10 to 0.90 $\mu\text{A}/\text{cm}^2$ with the polarization potential increased from 700 to 1000 mV. The current density increases quickly when the polarization potential is above 800 mV. The higher value of the corrosion current density or lower value of the R_p means that the oxidation kinetics is higher during dissolution. From potentiostatic polarization and Tafel polarization, it was concluded that the passive film-covered chalcopyrite obtained after electrochemical treatment in the potential window of 800–1000 mV dissolved faster. However, oxidation of the passive film-covered chalcopyrite obtained after electrochemical treatment in the potential window of 475–700 mV is slow. Thus, the passive film that formed in the potential window of 475–700 mV will hinder further dissolution of chalcopyrite. In the potential region of 800–1000 mV, accumulation of the product of oxidized chalcopyrite on the surface also has a significant effect on chalcopyrite dissolution.

Fig. 3.7C shows that when the chalcopyrite electrode was polarized at 700 mV for 30 min, the corrosion current density was lowest, only 0.10 $\mu\text{A}/\text{cm}^2$; therefore, EIS of chalcopyrite electrodes polarized at 700 mV for 30 min were further investigated. EIS tests were conducted at 700 mV. The frequency range was 10^{-2} to 10^5 Hz with an amplitude of 5 mV. ZSimpWin 3.10 software was used to fit the impedance data. The Bode plots and Nyquist impedance spectra of the passive film-covered chalcopyrite in iron-free and iron-containing (0.05 mol/L $\text{Fe}_2[\text{SO}_4]_3$ and 0.025 mol/L FeSO_4) solution is shown in Figs. 3.8 and 3.9, respectively.

The i – t curves in Fig. 3.5 show that a passive film formed on the chalcopyrite electrode surface; hence, the electrode processes of the passive film-covered chalcopyrite contained the oxidation of passive film at the solution–passive film interface whose rate depends on electrode potential E , and the oxidation of chalcopyrite at the passive film–chalcopyrite interface (in other words the passive film forms at the passive film–chalcopyrite interface) whose rate depends on electrode potential E the thickness of passive film l . And the thickness of passive film l varies with electrode potential E . Therefore, the Faradaic admittance of passive film oxidation is:

$$Y_1 = \frac{1}{R_1} \quad (3.17)$$

Based on Cao's expression (Cao, 1990), the Faradaic admittance of chalcopyrite oxidation or passive film formation is:

$$Y_2 = \frac{1}{R_2} + \frac{B}{a + j\omega} \quad (3.18)$$

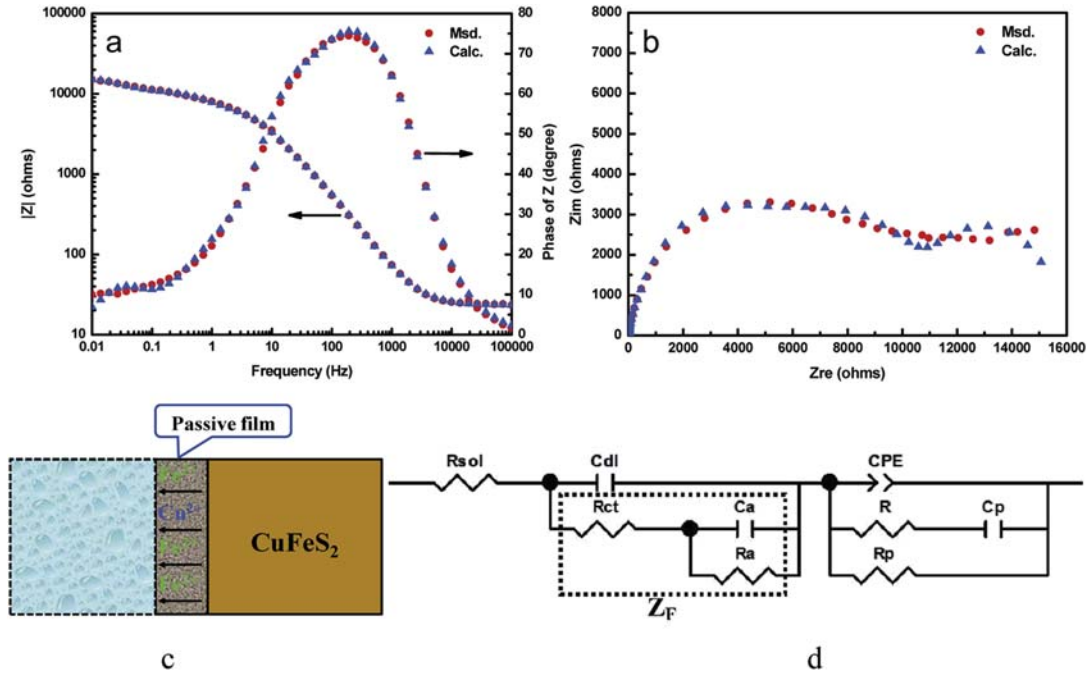


FIGURE 3.8 Bode plots (A), Nyquist impedance spectra (B), scheme of electrode process (C), and equivalent circuit (D) of a passive film-covered chalcopyrite electrode in an iron-free solution. *Msd.*, measured value; *Calc.*, calculated value; R_{sol} , solution resistance; C_{dl} , double-layer capacitance; R_{ct} , charge transfer resistance; C_a , equivalent capacitance; R_a , equivalent resistance; Z_F , faradaic impedance; CPE , constant phase element; R , resistance of constant phase element; C_p , capacitance of the passive layer; R_p , resistance of passive layer.

The total Faradaic admittance of the electrode processes of the passive film-covered chalcopyrite is:

$$Y_F = Y_1 + Y_2 = \frac{1}{R_{ct}} + \frac{B}{a + j\omega} \left(R_{ct} = \frac{R_1 R_2}{R_1 + R_2} \right) \quad (3.19)$$

where:

$$\frac{1}{R_1} = \left(\frac{\partial I_1}{\partial E} \right) \quad (3.20)$$

$$\frac{1}{R_2} = \left(\frac{\partial I_2}{\partial E} \right) \quad (3.21)$$

$$B = mb \quad (3.22)$$

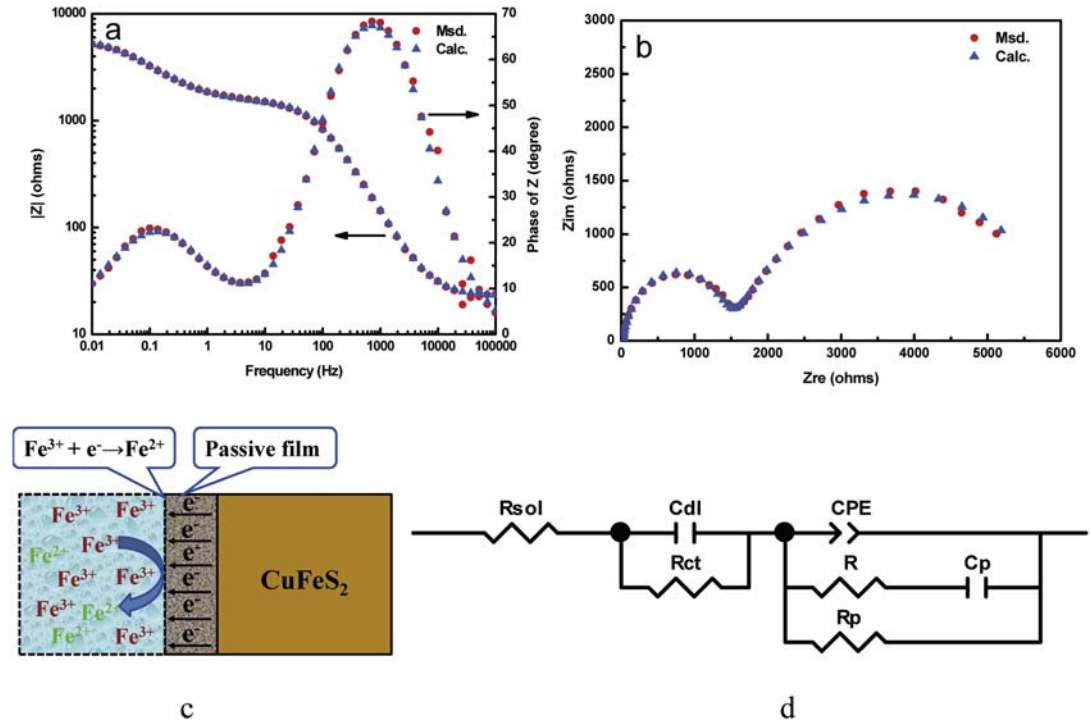


FIGURE 3.9 Bode plots (A), Nyquist impedance spectra (B), scheme of the electrode process (C), and equivalent circuit (D) of a passive film-covered chalcopyrite electrode in a ferric-containing solution. *Msd.*, measured value; *Calc.*, calculated value; R_{sol} , solution resistance; C_{dl} , double-layer capacitance; R_{ct} , charge transfer resistance; *CPE*, constant phase element; R , resistance of constant phase element; C_p , capacitance of the passive layer; R_p , resistance of passive layer.

$$m = \left(\frac{\partial I_2}{\partial I} \right)_E \quad (3.23)$$

$$b = \left(\frac{\partial [I]}{\partial E} \right)_I \quad (3.24)$$

$$[I] = \frac{dl}{dt} \quad (3.25)$$

$$a = - \left(\frac{\partial [I]}{\partial l} \right)_E \quad (3.26)$$

Many authors have claimed that Fe atoms dissolve preferentially from the chalcopyrite lattice during anodic dissolution and a passive layer forms on the surface (Chahremaninezhad, Dixon, & Asselin, 2013; Majuste, Ciminelli, Osseo-Asare, Dantas, & Magalhães-Paniago, 2012;

Nava & González, 2006; Yin, Kelsall, Vaughan, & England, 1995). The diffusion of Cu^{2+} and Fe^{2+} (mostly Fe^{2+}) from the chalcopyrite–passive film interface to the passive film–solution interface causes I_2 , defined as (Mott, 1947; Vetter, 1971):

$$I_2 = I_{20} \exp\left(\frac{\alpha z F d}{RT} \times \frac{V}{I}\right) \quad (3.27)$$

where F , R , T , V , z , d , and I represent the Faraday constant, ideal gas constant, temperature in Kelvin, potential difference between the solution–passive film interface and passive film–chalcopyrite interface, charge number of the migrating ion, jump distance of the migrating ion, and thickness of the passive film, respectively, and α , usually 0.5, is similar to a charge-transfer coefficient.

Therefore:

$$\frac{1}{R_2} = \frac{\alpha z F d}{RT I} \cdot I_2 \quad (3.28)$$

$$[I] = k(I_2 - I_1) \quad (3.29)$$

$$a = -k \left(\frac{\partial I_2}{\partial I} \right)_E = k \frac{\alpha z F d}{RT} \cdot \frac{V}{I^2} \cdot I_2 > 0 \quad (3.30)$$

$$m = -\frac{a}{k} \quad (3.31)$$

$$b = \left(\frac{\partial [I]}{\partial E} \right)_I = k \left(\frac{1}{R_2} - \frac{1}{R_1} \right) \quad (3.32)$$

$$B = a \left(\frac{1}{R_1} - \frac{1}{R_2} \right) \quad (3.33)$$

An expanded view of the potentiostatic polarization of chalcopyrite at 700 mV (Fig. 3.5) showed that the current density decreases slowly with prolonging polarization time, indicating that the dissolution rate of the passive layer (I_1) is less than the dissolution rate of chalcopyrite or the formation rate of passive film (I_2). Hence $B < 0$, thus the faradaic impedance (Z_F) can be equivalently shown as:

$$Z_F = \frac{1}{Y_F} = R_{ct} + \frac{R_a}{1 + j\omega R_a C_a} \quad (3.34)$$

where:

$$R_a = \frac{R_{ct}^2 |B|}{a - R_{ct} |B|} \quad (3.35)$$

$$C_a = \frac{1}{R_{ct}^2 |B|} \quad (3.36)$$

R_a and C_a are equivalent resistance and capacitance, respectively, associated with the thickness of passive film l .

Fig. 3.8A and B presents the Bode plots and Nyquist impedance spectra of the passive film-covered chalcopyrite in iron-free. The scheme of the electrode process and equivalent circuit used to fit the measured impedance spectra is presented in Fig. 3.8C and D, where C_{dl} is the double-layer capacitance, R_{sol} is the solution resistance, Z_F in the dotted rectangle is the circuit of faradaic impedance, R_{ct} is the charge transfer resistance of the electrode processes, C_a and R_a are equivalent capacitance and resistance, respectively, associated with the thickness of passive film l , C_p and R_p are the capacitance and resistance of the passive layer, and CPE and R are the constant phase element and its associated resistance, respectively, which represent the effect of crystal defects (Chen et al., 2008; Ghahremaninezhad, Dixon, & Asselin, 2012), surface heterogeneities and roughness, variation in coating composition, and electrode porosity (Jorcin et al., 2006). The fitted values of the electrical parameters are given in Table 3.1. The low chi-squared values given in Table 3.1 indicate a good fit with the experimental data.

Fig. 3.9A and B presents the Bode plots and Nyquist impedance spectra of the passive film-covered chalcopyrite in an iron-containing solution. In the iron-containing solution, ferric reduction (Eq. 3.37) contributes significantly to overall electrochemical processes occurring on the passive film-covered chalcopyrite electrode (Ghahremaninezhad et al., 2012);

TABLE 3.1 Impedance parameters for equivalent circuit.

	Solution resistance	Double-layer capacitance	Charge transfer resistance	Capacitance (C_a)	Resistance (R_a)	Y_0 , constant phase element
Elements	($\Omega \cdot \text{cm}^2$)	($\text{F} \cdot \text{cm}^{-2}$)	($\Omega \cdot \text{cm}^2$)	($\text{F} \cdot \text{cm}^{-2}$)	($\Omega \cdot \text{cm}^2$)	($\text{S} \cdot \text{cm}^{-2} \cdot \text{s}^n$)
Iron-free	23.37	3.59×10^{-6}	1773	4.03×10^{-6}	2604	4.13×10^{-5}
Iron-containing	22.79	3.44×10^{-4}	852.9	—	—	2.98×10^{-6}
Elements	n	Resistance ($\Omega \cdot \text{cm}^2$)	C_p ($\text{F} \cdot \text{cm}^{-2}$)	R_p ($\Omega \cdot \text{cm}^2$)	χ^2	
Iron-free	0.8	1.99×10^4	1.89×10^{-4}	1.15×10^4	8.60×10^{-4}	
Iron-containing	0.89	2388	5.16×10^{-4}	4398	3.81×10^{-3}	

hence, the scheme of the electrode process and equivalent circuit presented in Fig. 3.9C and D was chosen to model the measured impedance spectra, in which the elements are the same as before and the fitted values of the electrical parameters are given in Table 3.1:



From the R_{ct} values given in Table 3.1, the reduction of ferric ($R_{ct} = 852.9 \, \Omega \text{ cm}^2$) proceeds faster than the oxidation of chalcopyrite ($R_{ct} = 1773 \, \Omega \text{ cm}^2$), which means that the passive film is able to transfer electrons at a higher rate than ions. Comparisons of R_p values given in Table 3.1 further prove that the passive film is able to transfer electron more easily. In general, a passivation layer on the material surface hinders the dissolution rate owing to its low electrons or ionic conduction (Ghahremaninezhad et al., 2012). Hence, the passive film hinders the dissolution of chalcopyrite because of low ionic conduction.

3.2 Surface species of chalcopyrite after treatment by different potentials

XPS was employed to investigate the surface components of chalcopyrite electrodes after potentiostatic polarization in an electrolyte solution at 475, 600, 700, 800, 900, and 1000 mV for 1800 s. XPS was conducted with an ESCALAB 250Xi spectrometer with monochromatic Al excitation (1486.6 eV) operating at 200 W. Simultaneously, a flood-gun was used for charge compensation. High-resolution spectra were collected with a pass energy of 20 and 0.1 eV steps. All samples were run at ambient temperature and a chamber pressure of 1×10^{-9} mbar. Binding energy calibration was based on C 1s at 284.6 eV. The high-resolution S 2p spectra were fitted with a summed Gaussian (70%)–Lorentzian (30%) function and Shirley background.

Fig. 3.10 presents the XPS of Cu 2p, Cu LMM, S 2p Fe 2p, and O 1s for polished chalcopyrite. The fitting results of peaks revealed that the Cu 2p_{3/2} peak of chalcopyrite is centered at 932.2 eV (Fig. 3.10A). The Cu LMM is centered at 568.9 eV (Fig. 3.10B). This value is in agreement with the literature (Ghahremaninezhad et al., 2013).

Fig. 3.10C presents the S 2p peak of chalcopyrite, consisting of three major doublets; the S 2p_{3/2} peaks are centered at 161.3, 161.9, and 162.8 eV, respectively. The prominent S 2p_{3/2} peak centered at 161.3 eV originates from S²⁻ in bulk chalcopyrite (Ghahremaninezhad, Dixon, & Asselin, 2013; Nava, González, Leinen, & Ramos-Barrado, 2008). The low-intensity S 2p_{3/2} peaks centered at 161.9 and 162.8 eV is assigned to S₂²⁻ (Acres, Harmer, & Beattie, 2010b; Klauber, 2003) and S_n²⁻ (Acres, Harmer, & Beattie, 2010a, 2010b), respectively. In addition, a low-intensity 2p peak component at 164.3 eV can be ascribed to energy loss owing to S 3p → Fe 3d excitation (Acres, Harmer, & Beattie, 2010b; Klauber, 2003).

The high-resolution spectrum of Fe 2p for chalcopyrite exposed to air for no more than a few minutes is provided in Fig. 3.10D. The Fe 2p spectrum shows that even chalcopyrite exposed to air for only a few minutes was oxidized. The peak near 708 eV can be assigned to Fe–S (Ghahremaninezhad et al., 2013). The peak in the binding energy range of 710–712 eV can be assigned to Fe–O and Fe–OH (Li et al., 2014). This can be proved by the O 1s spectrum, which can be fitted with O²⁻, OH⁻ and chemisorbed H₂O (Li et al., 2014) (Fig. 3.10E). At the same time, Goh et al. (2006) suggested that the presence of O on

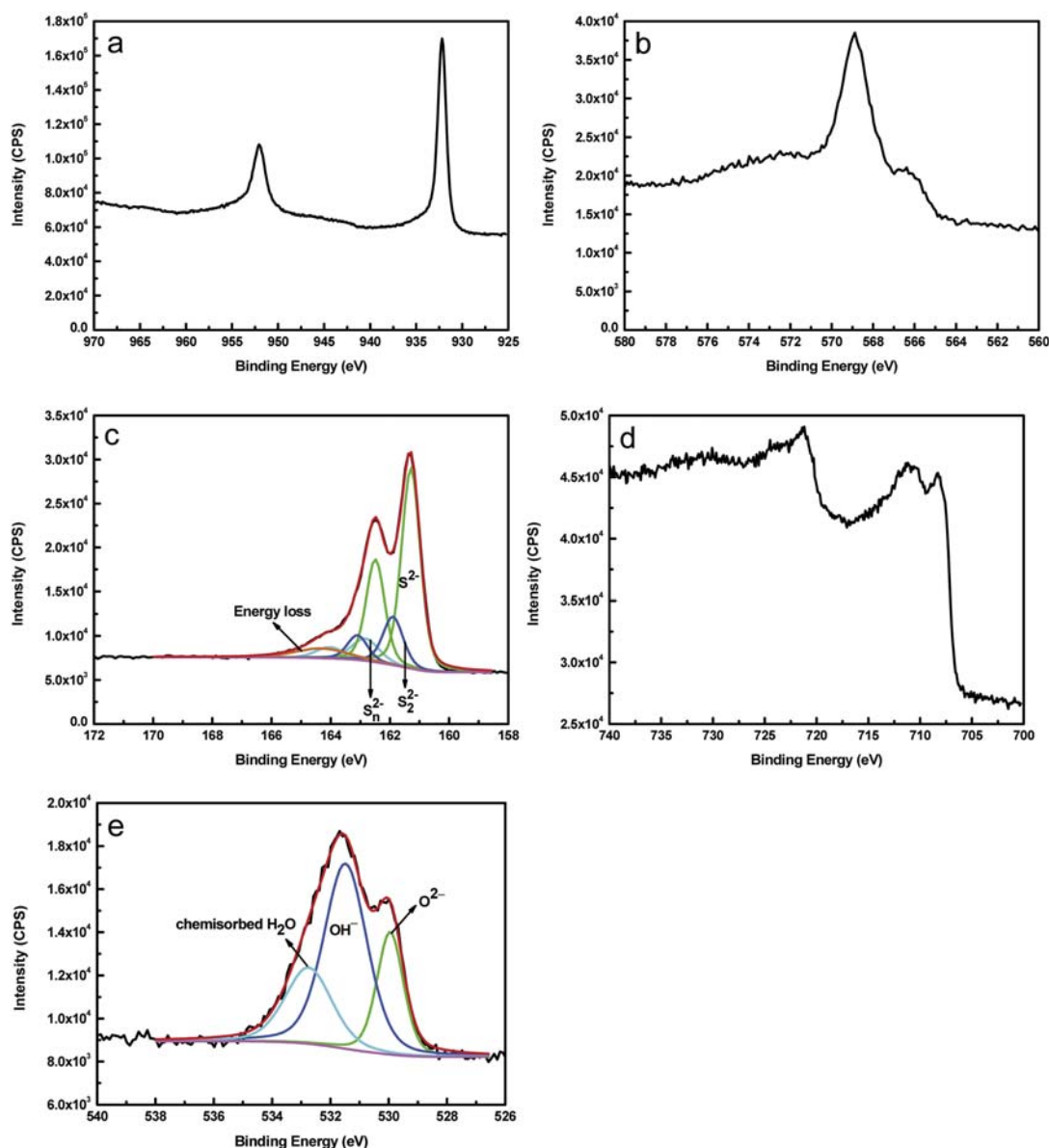


FIGURE 3.10 X-ray photoelectron spectroscopy spectrum of Cu 2p peak (A), Cu LMM peak (B), S 2p peak (C), and Fe 2p peak (D), and O 1s peak (E) of polished chalcopyrite. From Wu, S., Yang, C., Qin, W., Jiao, F., Wang, J., & Zhang, Y. (2015). Sulfur composition on surface of chalcopyrite during its bioleaching at 50°C. *Transactions of Nonferrous Metals Society of China*, 25(12), 4110–4118. [https://doi.org/10.1016/S1003-6326\(15\)64062-6](https://doi.org/10.1016/S1003-6326(15)64062-6).

the chalcopyrite surface was consistent with Fe–O compared with Cu–O species. According to Li et al. (2014), the formation of Fe–O is due to O_2 adsorption on Fe sites with subsequent dissociation, and the formation of Fe–OH is due to H_2O adsorption on Fe sites with subsequent dissociation. Furthermore, De Lima et al. (2011) suggested that water molecules

and the OH group absorb preferentially on iron atoms of reconstructed chalcopyrite (001) sulfur-terminated surfaces via the Fe–O bond. In addition, similar outcomes confirm that on the pyrite (FeS₂) surface, water molecule adsorption on the FeS₂ surface is via the Fe–O bond (Chen, Long, & Chen, 2014; Stirling, Bernasconi, & Parrinello, 2003). Mishra and Osseo-Asare (1988) stated that the opening step of pyrite oxidation in an aqueous solution is an adsorption OH group on iron sites of a pyrite surface. Therefore, O²⁻, OH⁻ and chemisorbed H₂O most probable adsorb on iron sites of the chalcopyrite surface. Moreover, binding energy near 712.5 eV would most probably be a Cu LMM peak (Ghahremaninezhad, Dixon, & Asselin, 2013; Harmer, Pratt, Nesbitt, & Fleet, 2005).

Fig. 3.11A and B shows Cu 2p and LMM photoelectron spectra obtained for a chalcopyrite electrode after electrochemical treatment at 475, 600, 700, 800, 900, and 1000 mV for 30 min. The binding energies for Cu 2p_{3/2} and Cu LMM are summarized in Table 3.2. It is well-established in the literature that there are two main XPS characteristics of Cu(II): the Cu 2p_{3/2} peak with a binding energy of 933.0–933.8 eV and the presence of satellite features around the binding energy of 942 eV, whereas a lower Cu 2p_{3/2} binding energy of 931.8–933.1 eV and the absence of satellite features are characteristics of Cu(I) and metal Cu (Chusuei, Brookshier, & Goodman, 1999; Ghahremaninezhad, Dixon, & Asselin, 2013; Goh, Buckley, Lamb, Rosenberg, & Moran, 2006; Yin et al., 2005). There is no known report of an Auger parameter less than 1850.5 eV for a Cu(II) compound (Goh et al., 2006). From Fig. 3.11A and B and Table 3.2, it can be seen that Cu on the surface of chalcopyrite after electrochemical treatment at different potentials is still +1. Cu₅FeS₄ (Majuste et al., 2012), CuS (Ghahremaninezhad, Dixon, & Asselin, 2013; Majuste, Ciminelli, Osseo-Asare, Dantas, & Magalhães-Paniago, 2012), CuS₂ (Yin et al., 1995), and Cu_{1-x}Fe_{1-y}S_{2-z} (Ghahremaninezhad, Dixon, & Asselin, 2013; Majuste, Ciminelli, Osseo-Asare, Dantas, & Magalhães-Paniago, 2012; Mikhlin et al., 2004; Nava & González, 2006) are suggested to be products of chalcopyrite electrochemical dissolution. Although the 2p_{3/2} binding energies for Cu in CuFeS₂ (Brion, 1980; Ghahremaninezhad, Dixon, & Asselin, 2013; Laajalehto, Kartio, & Nowak, 1994; Nakai, Sugitani, Nagashima, & Niwa, 1978), Cu₅FeS₄ (Goh, Buckley, Lamb, Rosenberg, & Moran, 2006; Harmer, Pratt, Nesbitt, & Fleet, 2005), and CuS (Brion, 1980; Chawla, Sankarraman, & Payer, 1992; Ghahremaninezhad, Asselin, & Dixon, 2011; Jiang et al., 2005) are close, it is difficult to use the Cu 2p binding energy and Cu LMM (Table 3.2) to identify which compounds are present.

Fig. 3.11C and D presents the Fe 2p photoelectron spectrum obtained for chalcopyrite electrodes after electrochemical treatment at 475, 600, 700, 800, 900, and 1000 mV for 30 min. When the polarization potential is less than 800 mV, Fe 2p peaks are similar. However, when the polarization potential is above 800 mV, the value of the Fe 2p peak shifted to a higher binding energy. The peak near 708 eV can be assigned to Fe–S (Ghahremaninezhad et al., 2013). The peak in the binding energy range of 710–712 eV can be assigned to Fe(III) –O, –OH, or –OOH (Acres, Harmer, & Beattie, 2010b; Brion, 1980; Ghahremaninezhad, Dixon, & Asselin, 2013; Harmer, Thomas, Fornasiero, & Gerson, 2006). The component near the 712.5 eV binding energy would most probably be a Cu LMM peak (Ghahremaninezhad, Dixon, & Asselin, 2013; Harmer, Pratt, Nesbitt, & Fleet, 2005).

Fig. 3.11E and F shows a series of S 2p and S 2s photoelectron spectra obtained for chalcopyrite electrodes after electrochemical treatment at 475, 600, 700, 800, 900, and 1000 mV for 30 min. The fitted photoelectron spectra of S 2p peaks of Fig. 3.11E are presented in Fig. 3.12 and Table 3.3.

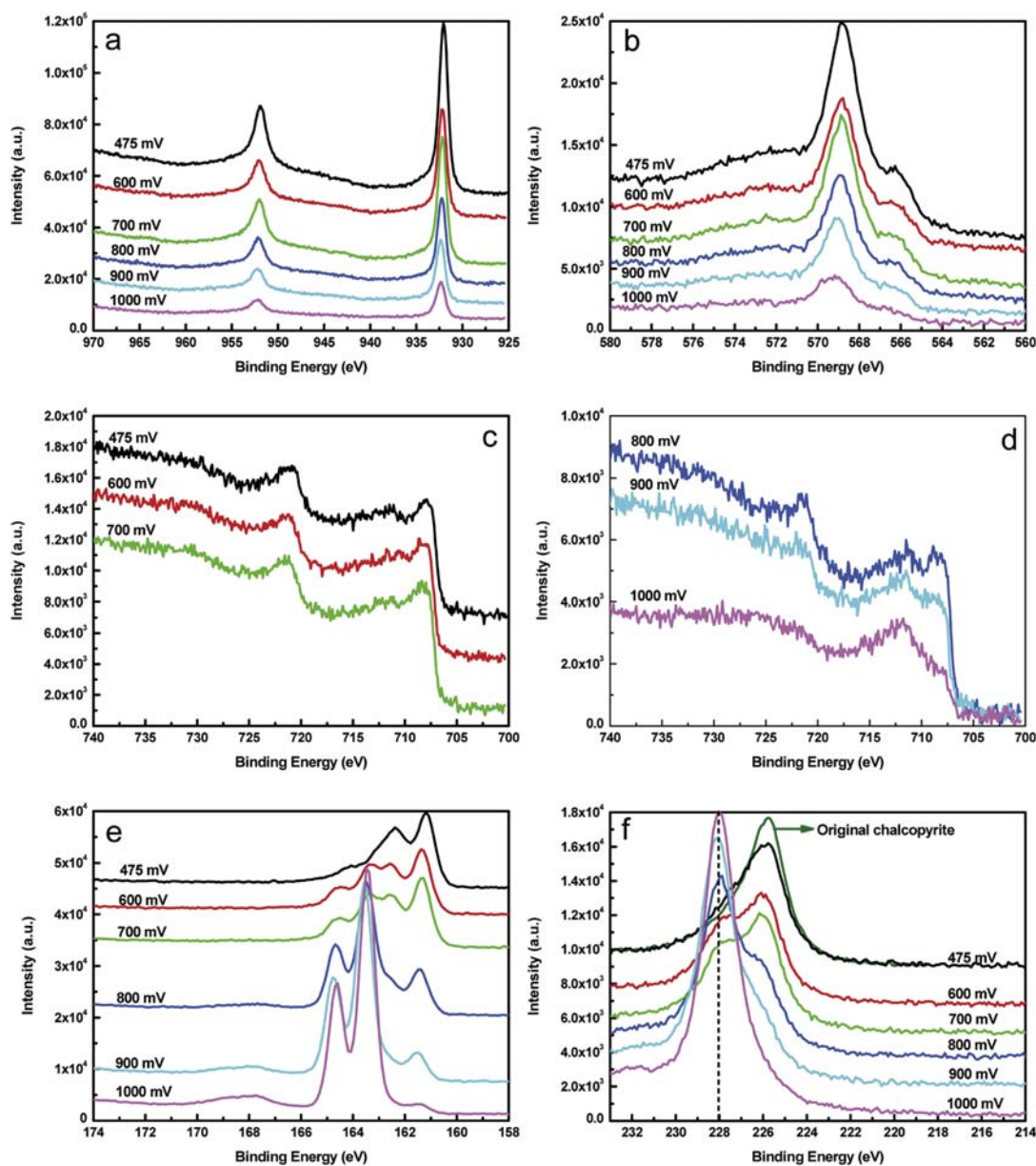


FIGURE 3.11 Comparison of Cu 2p (A), Cu LMM (B), Fe 2p (C, D), S 2p (E), and S 2s (F), and peaks of chalcopyrite after dissolution at 475, 600, 700, 800, 900, and 1000 mV for 30 min. From Yang, C., Qin, W., Zhao, H., Wang, J., & Wang, X. (2018). Mixed potential plays a key role in leaching of chalcopyrite: Experimental and theoretical analysis. *Industrial and Engineering Chemistry Research*, 57(5), 1733–1744. <https://doi.org/10.1021/acs.iecr.7b02051>.

TABLE 3.2 Binding energy values for X-ray photoelectron spectroscopy spectra of Cu 2p_{3/2} and kinetic energy values for Cu LMM peaks.

Condition	Cu 2p _{3/2}		Cu LMM	Auger parameter
	Binding energy (eV)	FWHM	Kinetic energy (eV)	(eV)
CuFeS ₂	932.2	1.1	917.7	1849.9
475 mV	932.1	1.1	917.8	1849.9
600 mV	932.2	1.1	917.8	1850
700 mV	932.2	1.1	917.8	1850
800 mV	932.3	1.1	917.7	1850
900 mV	932.4	1.2	917.6	1850
1000 mV	932.4	1.2	917.4	1849.8

Data from Yang, C., Qin, W., Zhao, H., Wang, J., & Wang, X. (2018). Mixed potential plays a key role in leaching of chalcopyrite: Experimental and theoretical analysis. *Industrial and Engineering Chemistry Research*, 57(5), 1733–1744. <https://doi.org/10.1021/acs.iecr.7b02051>.

The S 2p_{3/2} binding energies of chalcopyrite after electrochemical treatment at different potentials are presented in Table 3.3. The S 2p_{2/3} binding energies in the range of 161.2–161.5 eV are in agreement with S^{2−} (Acres, Harmer, & Beattie, 2010a, 2010b; Gerson & Bredow, 2000; Ghahremaninezhad, Dixon, & Asselin, 2013; Goh, Buckley, & Lamb, 2006; Nava, González, Leinen, & Ramos-Barrado, 2008). The S 2p_{2/3} peak with the binding energy of 161.9–162.2 eV is in agreement with the S 2p_{3/2} peak position of S₂^{2−} (Acres, Harmer, & Beattie, 2010a, 2010b; Ghahremaninezhad, Dixon, & Asselin, 2013; Goh, Buckley, & Lamb, 2006; Klauber, 2003), and the S 2p_{2/3} peak with the binding energy of 162.8–163.2 eV agrees with the S 2p_{3/2} peak position of S_n^{2−} (Acres, Harmer, & Beattie, 2010a, 2010b; Ghahremaninezhad, Dixon, & Asselin, 2013).

The S 2p_{2/3} peak with the binding energy of 163.5–163.8 eV (Fig. 3.12 and Table 3.3) is in agreement with the S 2p_{3/2} peak position of S⁰ (Gerson & Bredow, 2000; Sasaki, Takatsugi, Ishikura, & Hirajima, 2010; Smart, Skinner, & Gerson, 1999). It has been well-established in the literature that the S 2s peaks of the S atoms in chalcopyrite, covellite, and chalcocite are 225.6, 225.6, and 225.3 eV, respectively, whereas the S 2s peak of elemental sulfur occurs at a binding energy of 228.4 eV (Ghahremaninezhad et al., 2013). Therefore, the S 2s peak can be used to detect the formation of elemental sulfur. Fig. 3.11F shows that the S 2s binding energy shifts to higher energies within an increase in potential from 475 to 1000 mV; indicates elemental sulfur formation on the chalcopyrite surface.

Elemental sulfur is generally considered a product of CuFeS₂ dissolution (Eqs. 3.38–3.39) (Farquhar, Wincott, Wogelius, & Vaughan, 2003; Ghahremaninezhad, Dixon, & Asselin, 2013; Majuste, Ciminelli, Osseo-Asare, Dantas, & Magalhães-Paniago, 2012) and/or the product of CuS (Eq. 3.40) dissolution at high potentials ($E \geq 0.8$ V) (Ghahremaninezhad, Dixon, & Asselin, 2013; Majuste, Ciminelli, Osseo-Asare, Dantas, & Magalhães-Paniago, 2012; Nava & González, 2006), which was considered the product of chalcopyrite dissolution (Eq. 3.39)

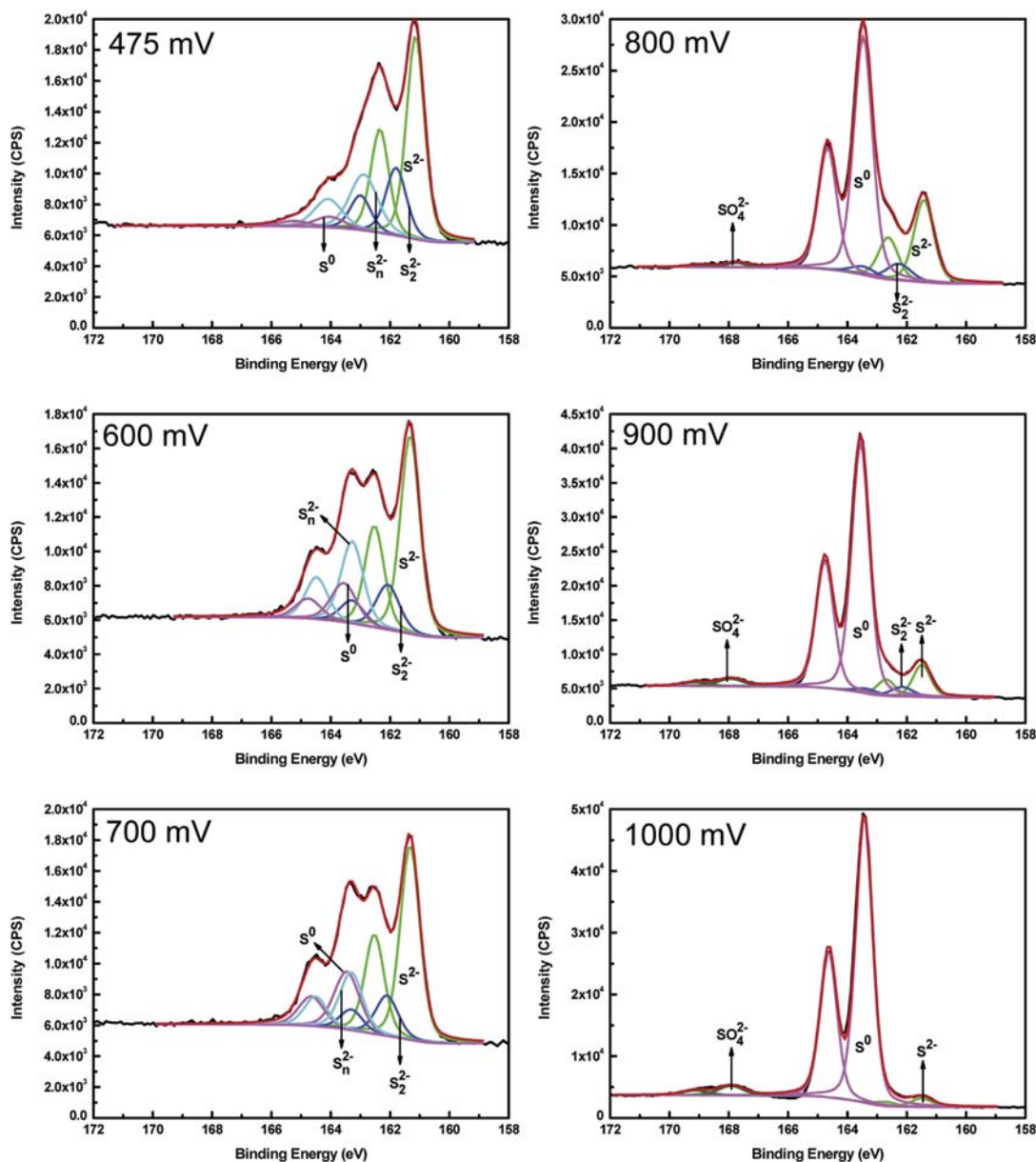


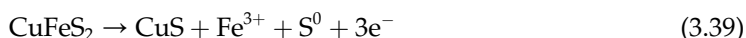
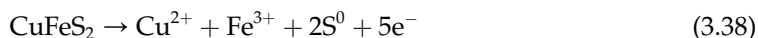
FIGURE 3.12 S 2p X-ray photoelectron spectroscopy spectra for chalcopyrite electrode surface obtained after dissolution at 475, 600, 700, 800, 900, and 1000 mV for 30 min. From Yang, C., Qin, W., Zhao, H., Wang, J., & Wang, X. (2018). Mixed potential plays a key role in leaching of chalcopyrite: Experimental and theoretical analysis. *Industrial and Engineering Chemistry Research*, 57(5), 1733–1744. <https://doi.org/10.1021/acs.iecr.7b02051>.

TABLE 3.3 Binding energy values for X-ray photoelectron spectroscopy spectra of S 2p_{3/2} peaks (eV).

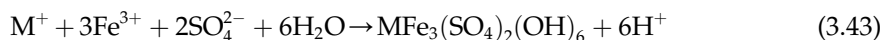
Condition	Monosulfide	Disulfide	Polysulfide	Elemental sulfur	Sulfate	Energy loss
CuFeS ₂	161.3	161.9	162.8			164.3
475 mV	161.2	162	163	163.8		
600 mV	161.3	162.2	163.2	163.7		
700 mV	161.3	162.2	163.2	163.6		
800 mV	161.4	162.2		163.5	167.8	
900 mV	161.5	162.2		163.6	167.9	
1000 mV	161.5			163.5	167.9	

Data from Yang, C., Qin, W., Zhao, H., Wang, J., & Wang, X. (2018). Mixed potential plays a key role in leaching of chalcopyrite: Experimental and theoretical analysis. *Industrial and Engineering Chemistry Research*, 57(5), 1733–1744. <https://doi.org/10.1021/acs.iecr.7b02051>.

(Majuste, Ciminelli, Osseo-Asare, Dantas, & Magalhães-Paniago, 2012; Nava & González, 2006):



When the applied potential is above 800 mV, the S 2p_{2/3} binding energy centered at about 167.9 eV (Fig. 3.12 and Table 3.3) is related to SO₄²⁻ (Ghahremaninezhad et al., 2013), most probably due to sulfate formation (Eqs. 3.41–3.43). This peak was not detected on the surface of chalcopyrite polarized at 475, 600, and 700 mV (Fig. 3.12 and Table 3.3):



Fe preferentially dissolves from the chalcopyrite surface in potentials ranging from 475 to 700 mV, leaving behind an S₂²⁻ and S_n²⁻ passive film. The passive film consisting of S₂²⁻ and S_n²⁻ was oxidized when the applied potential was higher than 700 V, and transpassive dissolution of chalcopyrite occurred.

3.3 Disulfide and polysulfide passivation of the chalcopyrite surface

The low leaching rate of chalcopyrite results from passivation of the mineral surface. However, the composition of the passive film is still debated. Therefore, an understanding of chalcopyrite dissolution and surface composition is necessary to improve Cu extraction.

Fig. 3.13 shows Cu, Fe, and S fraction and sulfur species on the surface of chalcopyrite after 1800 s of electrochemical treatment at 475, 600, 700, 800, 900, and 1000 mV. To obtain the data in Fig. 3.13B, it was assumed that the percentage of each species (S^{2-} , S_2^{2-} , S_n^{2-} , S^0 , and SO_4^{2-}) is directly proportional to the area under its S 2p peak, presented in Fig. 3.12.

When the chalcopyrite electrode was polarized at 475 mV, Fe atoms dissolve preferentially than Cu atoms from the chalcopyrite lattice which leads to the increase in S and Cu fraction at the surface (Fig. 3.13A). Simultaneously, it leaves behind a S_2^{2-} and S_n^{2-} film (Fig. 3.13B).

When the polarization potential increases from 475 to 600 mV, the S fraction increases owing to the dissolution of Cu from the chalcopyrite surface, but the Fe fraction changes only slightly (Fig. 3.13A). When the polarization potential further increases from 600 to 700 mV, the S, Fe, and Cu fraction on the chalcopyrite surface is almost constant (Fig. 3.13A). Furthermore, the S^{2-} fraction on the chalcopyrite surface slightly decreases from 60% to 53% with an increase in the polarization potential from 475 to 600 mV (Fig. 3.13B); but the S^{2-} fraction on the chalcopyrite surface is constant (about 53%) in the potential range of 600–700 mV (Fig. 3.13B), which indicates that no more chalcopyrite dissolves with an increase in potential. In other words, a passivation film forms on the chalcopyrite surface in the potential range of 475–700 mV. In this potential range, in addition to S^{2-} , the most sulfur species on the chalcopyrite surface is S_n^{2-} , followed by S_2^{2-} . Therefore, S_2^{2-} and S_n^{2-} are considered to be composed of chalcopyrite passivation film. Ghahremaninezhad et al. (2012, 2013) suggested that $Cu_{1-x}Fe_{1-y}S_{2-z}$, which consists of Cu–S and Fe–S bonds, passivates the chalcopyrite surface owing to its low ionic conductivity when the

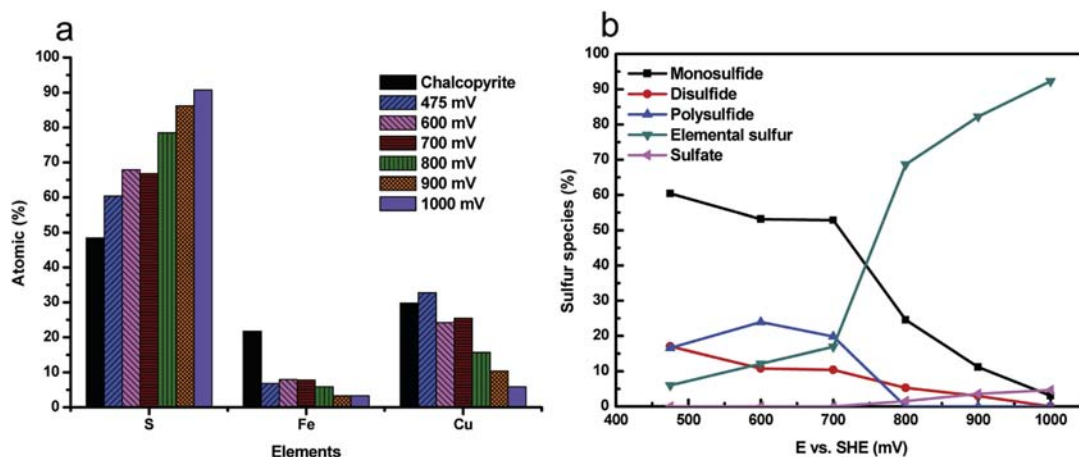
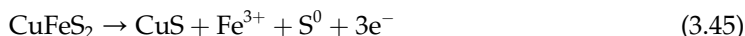
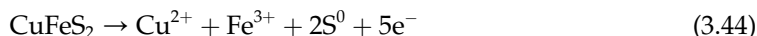


FIGURE 3.13 Composition of Cu, Fe, and S fraction (A) and sulfur species (B) on the surface of chalcopyrite after dissolution at 475, 600, 700, 800, 900, and 1000 mV for 1800 s. From Yang, C., Qin, W., Zhao, H., Wang, J., & Wang, X. (2018). Mixed potential plays a key role in leaching of chalcopyrite: Experimental and theoretical analysis. *Industrial and Engineering Chemistry Research*, 57(5), 1733–1744. <https://doi.org/10.1021/acs.iecr.7b02051>.

potential is below 0.90 V, but above 0.90 V, transpassive dissolution occurs. Simultaneously, S^0 and S_n^{2-} were detected on the surface. Yang et al. (2015) stated that the metal-deficient S_n^{2-}/S^0 layer passivates chalcopyrite anodic dissolution when the potential is lower than 530 mV (compared with Ag/AgCl); they concluded the same results during chalcopyrite bioleaching (Yang et al., 2014). However, Klauber (2008) argued that the physical reality of $Cu_{1-x}Fe_{1-y}S_{2-z}$ is questioned, and S_n^{2-} could not be considered passivation candidates because they were easily oxidized to S^0 upon exposure to air and water. However, more and more researchers detected S_n^{2-} on the chalcopyrite surface by XPS (Acres, Harmer, & Beattie, 2010b; Mikhlin et al., 2004; Von Oertzen, Harmer, & Skinner, 2006; Wu et al., 2015; H. Zhao et al., 2015; H.B. Zhao et al., 2015). It was also detected in our study. Furthermore, according to density functional theory calculations, S_4^{2-} was formed on the chalcopyrite surface (De Oliveira, De Lima, De Abreu, & Duarte, 2012; Von Oertzen, Harmer, & Skinner, 2006). Holmes and Crundwell (2013) and Crundwell (2013) suggested that S_n^{2-} and $Cu_{1-x}Fe_{1-y}S_{2-z}$ are essentially the same; the only difference is the focus on either sulfur or metal removal. However, Crundwell et al. (2015a, 2015b) argued that the rate of chalcopyrite dissolution is not limited by passivation films or layers, but is intrinsically slow owing to the semiconducting properties of chalcopyrite. Furthermore, they identified chalcopyrite anodic dissolution as a depletion region (0.3–0.6 V versus SCE) and inversion region (>0.7 V versus SCE) for ideal n-type semiconductor. In the depletion region, the current is limited by the depletion of electrons in the space-charge layer, and no reactions take place at the surface and supply electrons in this region. Fig. 3.13B shows that during the increase in potential from 475 to 600 mV, the fraction of S^{2-} and S_2^{2-} on the chalcopyrite surface decreases by $\sim 7\%$ and $\sim 6\%$, respectively, but the fraction of S_n^{2-} and S^0 increases by $\sim 7\%$ and $\sim 6\%$, respectively; furthermore, for an increase in potential from 600 to 700 mV, the fraction of S^{2-} and S_2^{2-} on the chalcopyrite surface is almost constant, but the S_n^{2-} is oxidized to S^0 . Therefore, the low current results from chalcopyrite surface passivation.

When the applied potential is above 700 V, the fraction of Cu and Fe on the chalcopyrite surface decreases with an increase in potential, whereas the fraction of S on the chalcopyrite surface increases with an increase in potential (Fig. 3.13A). Fig. 3.13B shows that when the applied potential is above 700 V, the fraction of S^{2-} , S_2^{2-} , and S_n^{2-} show an obvious decrease (S^{2-} decreases from 53% to 3%, S_2^{2-} decreases from 10% to 0%, and S_n^{2-} decreases from 20% to 0%). Moreover, S^0 increases from 17% to 92%. In addition, the concentration of SO_4^{2-} increases from 0% to 5%. When the applied potential is increased from 700 to 800 mV, the fraction of S_n^{2-} decreases from 20% to 0%. The fraction of S^{2-} decreases from 53% to 25%, whereas the S^0 shows a sudden increase (S^0 increases from 17% to 69%) indicating that the chalcopyrite dissolved. Elemental sulfur is generally considered a product of $CuFeS_2$ dissolution (Eqs. 3.44 and 3.45) (Farquhar, Wincott, Wogelius, & Vaughan, 2003; Ghahremaninezhad, Dixon, & Asselin, 2013; Majuste, Ciminelli, Osseo-Asare, Dantas, & Magalhães-Paniago, 2012), and/or the product of CuS (Eq. 3.46) dissolution at high potentials ($E \geq 0.8$ V) (Ghahremaninezhad, Dixon, & Asselin, 2013; Majuste, Ciminelli, Osseo-Asare, Dantas, & Magalhães-Paniago, 2012; Nava & González, 2006), which was detected by Majuste et al. (2012) on the surface of chalcopyrite after electrochemical dissolution at 0.80 V (Eq. 3.45). In the current study, when the polarization potential increased from 900 to 1000 mV, the increase in S fraction and decrease in Cu fraction were equivalent, and the Fe fraction did not change

(Fig. 3.13A). This indicates the presence of CuS on the chalcopyrite surface. SO_4^{2-} originates from jarosites formed on the chalcopyrite surface, according to Eq. (3.43):



In order to further certify that the S_2^{2-} and S_n^{2-} passivate the chalcopyrite surface, and the passive film would be oxidized when the potential is above 800 mV. Chalcopyrite was leached with 1 mol/L $\text{Fe}_2(\text{SO}_4)_3$, 3 mol/L HNO_3 , and 0.3 mol/L $\text{K}_2\text{Cr}_2\text{O}_7$, respectively. The redox potential of $\text{Fe}^{3+}/\text{Fe}^{2+}$, NO_3^-/NO , and $\text{Cr}_2\text{O}_7^{2-}/\text{Cr}^{3+}$ was 0.771, 0.957, and 1.232 V, respectively. Then, 3%, 83%, and 96% Cu were extracted after 24 h of leaching with $\text{Fe}_2(\text{SO}_4)_3$, HNO_3 , and $\text{K}_2\text{Cr}_2\text{O}_7$, respectively. XRD patterns of leaching residuals are shown in Fig. 3.14. Only the characteristic peaks of chalcopyrite were detected by XRD when chalcopyrite was leached with $\text{Fe}_2(\text{SO}_4)_3$. S^{2-} , S_2^{2-} , S_n^{2-} , and S^0 were observed by XPS on the surface of chalcopyrite (Fig. 3.15). When chalcopyrite was leached with HNO_3 , the characteristic peaks of chalcopyrite were detected by XRD, together with low-intensity characteristic peaks of elemental sulfur. However, XPS analysis showed that the main sulfur species on the surface of chalcopyrite was S^0 , together with low-intensity SO_4^{2-} (Figs. 3.15 and 3.16). Only the characteristic peaks of elemental sulfur were detected by XRD when chalcopyrite was leached with $\text{K}_2\text{Cr}_2\text{O}_7$. At the same time, S^0 and SO_4^{2-} was observed by XPS (Figs. 3.15 and 3.16). It can be concluded from this that S_2^{2-} and S_n^{2-} passivate the chalcopyrite surface, which cannot be

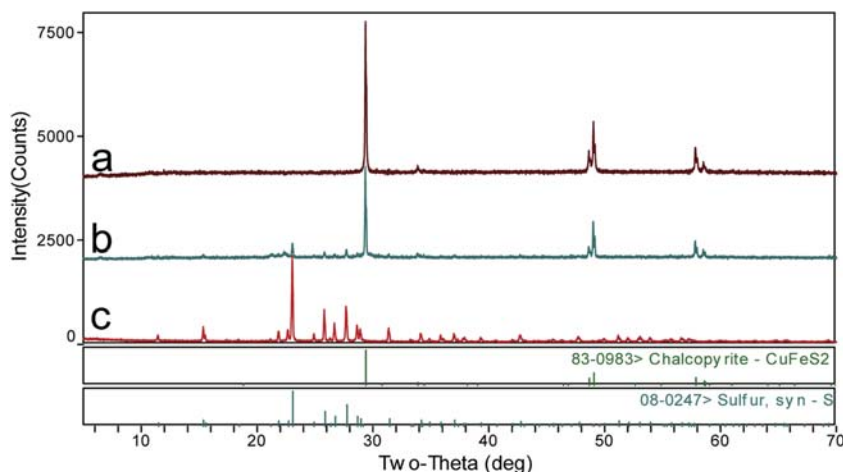


FIGURE 3.14 X-ray photoelectron spectroscopy patterns of chalcopyrite after dissolution in 1.0 mol/L sulfuric acid. (A) 1.0 mol/L $\text{Fe}_2(\text{SO}_4)_3$. (B) 3.0 mol/L HNO_3 . (C) 0.3 mol/L $\text{K}_2\text{Cr}_2\text{O}_7$. From Yang, C., Qin, W., Zhao, H., Wang, J., & Wang, X. (2018). Mixed potential plays a key role in leaching of chalcopyrite: Experimental and theoretical analysis. *Industrial and Engineering Chemistry Research*, 57(5), 1733–1744. <https://doi.org/10.1021/acs.iecr.7b02051>.

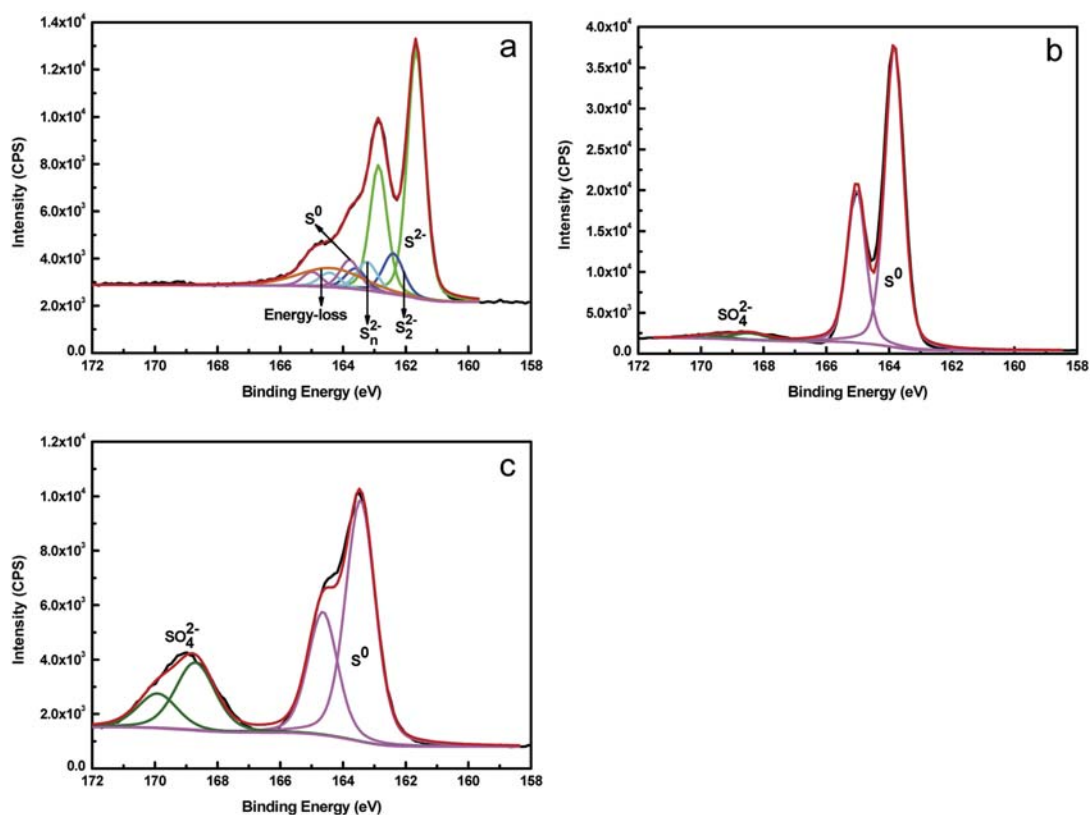


FIGURE 3.15 S 2p peaks of chalcoprite dissolution in 1.0 mol/L sulfuric acid. (A) 1.0 mol/L $\text{Fe}_2(\text{SO}_4)_3$. (B) 3.0 mol/L HNO_3 . (C) 0.3 mol/L $\text{K}_2\text{Cr}_2\text{O}_7$. From Yang, C., Qin, W., Zhao, H., Wang, J., & Wang, X. (2018). Mixed potential plays a key role in leaching of chalcoprite: Experimental and theoretical analysis. *Industrial and Engineering Chemistry Research*, 57(5), 1733–1744. <https://doi.org/10.1021/acs.iecr.7b02051>.

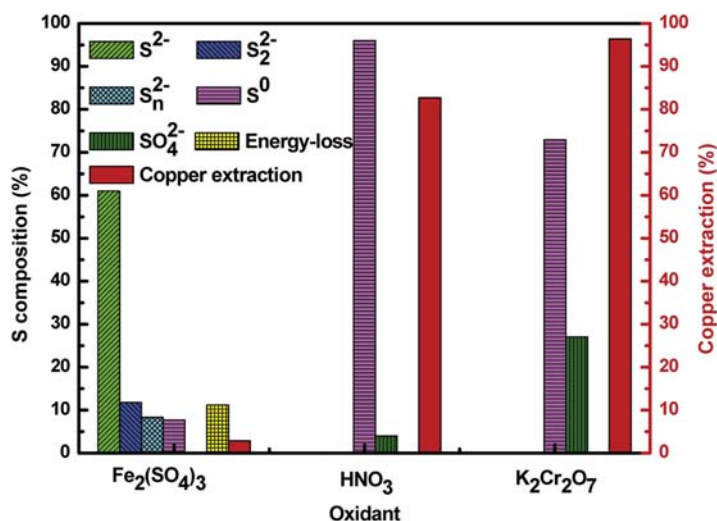


FIGURE 3.16 Sulfur species on the surface of chalcoprite and copper extraction. From Yang, C., Qin, W., Zhao, H., Wang, J., & Wang, X. (2018). Mixed potential plays a key role in leaching of chalcoprite: Experimental and Theoretical analysis. *Industrial and Engineering Chemistry Research*, 57(5), 1733–1744. <https://doi.org/10.1021/acs.iecr.7b02051>.

oxidized by Fe^{3+} . However, the passive film can be oxidized to S^0 by NO_3^- and $\text{Cr}_2\text{O}_7^{2-}$ owing to their higher redox potential; thus, higher copper extraction was obtained (Figs. 3.15 and 3.16). Furthermore, the presence of S^0 on the surface of the chalcopyrite does not hinder chalcopyrite leaching (Figs. 3.15 and 3.16). These are in agreement with electrochemical results showing that S_2^{2-} and S_n^{2-} passivate the chalcopyrite surface.

During leaching, Fe atoms dissolve more preferentially than Cu atoms from the chalcopyrite lattice, leaving behind an S-rich layer consisting of S_2^{2-} and S_n^{2-} . The S-rich layer is $\text{Cu}_{1-x}\text{Fe}_{1-y}\text{S}_2$ ($y \gg x$) and passivates the chalcopyrite surface. When metal atoms are released entirely from $\text{Cu}_{1-x}\text{Fe}_{1-y}\text{S}_2$ ($y \gg x$) and this fully depleted layer becomes thicker than several atomic layers, restructuring may occur to form S_8 -like S^0 .

3.4 Optimum range of redox potential for chalcopyrite leaching

The dissolution process for chalcopyrite during bioleaching involves complicated oxidation-reduction reactions, which vary with the changes in the compositions of the solution. Ferric ions act as the main oxidant in both chemical leaching (ferric leaching) and bioleaching (Klauber, 2008; Li, Kawashima, Li, Chandra, & Gerson, 2013; Watling, 2013). Redox potential has been proved to be an important and even dominating factor in chalcopyrite leaching by ferric sulfate (Córdoba, Muñoz, Blázquez, González, & Ballester, 2008a; Sandström, Shchukarev, & Paul, 2005). For the bioleaching of chalcopyrite, some researchers have proposed that the dissolution of chalcopyrite can be accelerated by controlling the redox potential at a relatively low value, and extremely high redox potentials at the initial stage of bioleaching can cause rapid passivation of chalcopyrite, inhibiting its further dissolution (Córdoba, Muñoz, Blázquez, González, & Ballester, 2008b; Gericke, Govender, & Pinches, 2010; Petersen & Dixon, 2006; Third, Cord-Ruwisch, & Watling, 2000, 2002). Petersen and Dixon (2006) claimed that a high redox potential favors the bioleaching of pyrite, whereas a low redox potential favors the bioleaching of chalcopyrite. Chalcopyrite leaching stops almost completely when the redox potential slips beyond the critical potential (at a ferric to ferrous ratio of about 1). Ahmadi et al. (2010) used an electro-bioreactor to control the solution potential in the bioleaching of chalcopyrite and discovered that chalcopyrite can be effectively extracted by controlling the redox potential at 400–425 mV (versus Ag/AgCl). Some researchers proposed that chalcopyrite can be reduced to chalcocite (Cu_2S) at a low redox potential and the formed chalcocite (Cu_2S) can be rapidly oxidized (Elsherief, 2002; Gu, Hu, Zhang, Xiong, & Yang, 2013; Hiroyoshi, Miki, Hirajima, & Tsunekawa, 2000, 2001; Third, Cord-Ruwisch, & Watling, 2002).

The dissolution process of chalcopyrite in bioleaching systems is mainly composed of complicated oxidation-reduction reactions dependent on oxidants and reductants of dissolved oxygen, Fe^{3+} and Fe^{2+} . Hence, the roles of O_2 , Fe^{3+} , and Fe^{2+} for bioleaching chalcopyrite is extremely important for understanding the mechanisms of chalcopyrite bioleaching, interpreting inconsistencies in chalcopyrite hydrometallurgy, and providing useful methods for promoting the bioleaching of chalcopyrite.

3.4.1 Fe^{2+} accelerating dissolution of chalcopyrite

Two grams of chalcopyrite with a particle size of 100% passing -0.074 mm was added to 250-mL flasks containing 100 mL of sulfuric acid solutions, pH 1.6, and target concentrations of Fe^{2+} and Fe^{3+} were added to the solution. Then, the flasks were placed in an orbital shaker at 160 rpm and 50°C . The solution pH was adjusted periodically to 1.6 with diluted sulfuric acid. Next, 1.0 mL of leaching solution was sampled and analyzed for Cu and Fe using inductively coupled plasma–atomic emission spectrometry (ICP-AES) (America Baird Co., PS-6). The sample volume was replaced with an equal volume of sulfuric acid solutions, pH 1.6. Water lost by evaporation was supplemented periodically by adding distilled water until the mass of the flask equaled its initial mass. After the leaching, the leaching residuals were washed with sulfuric acid solutions, pH 1.6, and dried in a vacuum box at 50°C . The dried leaching residuals were examined by XRD and Raman spectroscopy with a 633-nm He-Ne laser (Horiba Jobin Yvon Labram HR800).

Fig. 3.17 shows the copper extraction percentage, total iron concentration, and mixed potential during the leaching of chalcopyrite with different Fe^{2+} and Fe^{3+} concentrations.

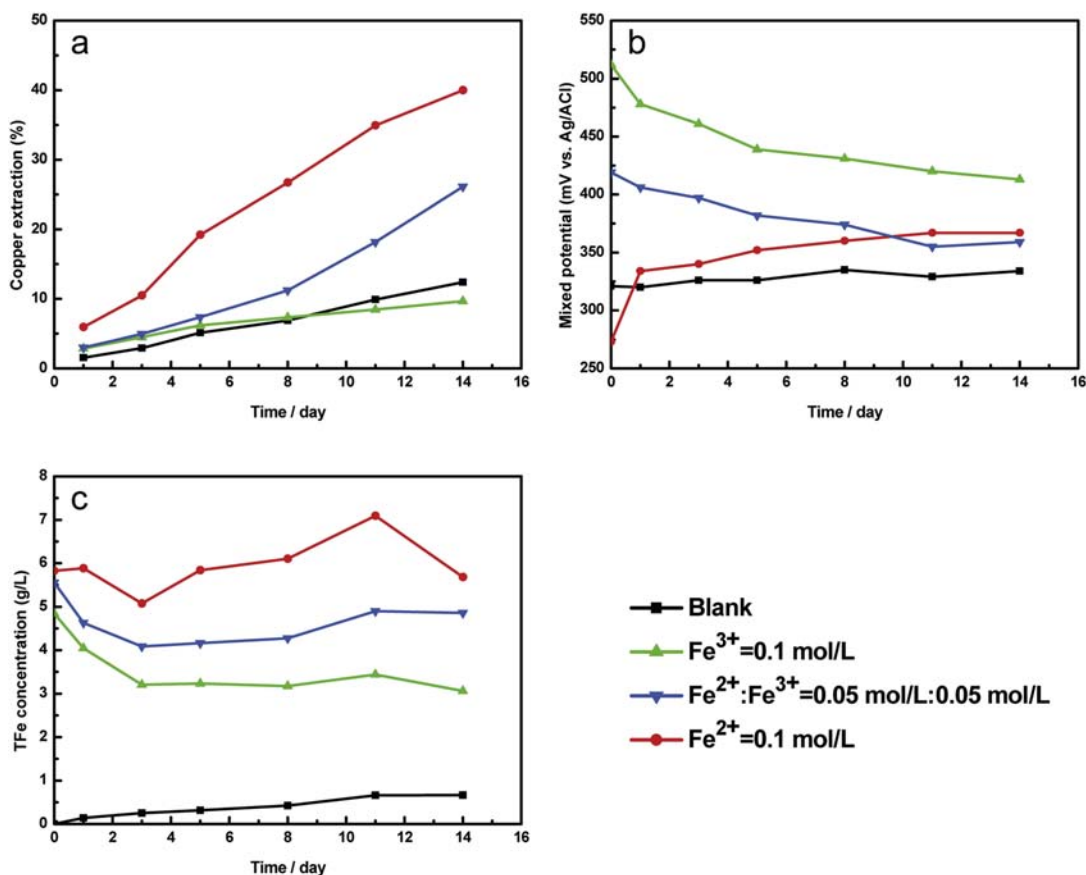


FIGURE 3.17 Effect of Fe^{2+} and Fe^{3+} ions on chemical leaching of chalcopyrite: (A) copper extraction, (B) mixed potentials, and (C) concentration of total dissolved iron. From Yang, C., Qin, W., Zhao, H., Wang, J., & Wang, X. (2018). *Mixed potential Plays a key role in leaching of chalcopyrite: Experimental and theoretical analysis*. Industrial and Engineering Chemistry Research, 57(5), 1733–1744. <https://doi.org/10.1021/acs.iecr.7b02051>.

In blank solutions at pH 1.6, the copper extraction percentage increased with time and reached 12% after 14 days. Similar to copper, the total iron concentration increased with time and reached 0.66 g/L after 14 days. During the whole leaching process, the mixed potential stabilized at about 330 mV; iron in the leaching solution presented as Fe^{2+} because the mixed potential of the sulfate leaching solution was decided by the ratio of Fe^{3+} to Fe^{2+} (Jones, 1974; Jones & Peters, 1976). The chalcopyrite dissolution in acid solutions is described in Eq. (3.47):

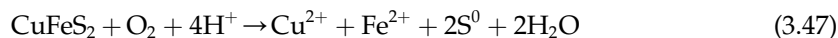
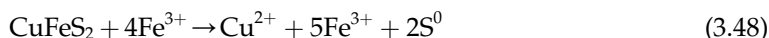


Fig. 3.17A shows that the percentage of copper extraction decreased by adding Fe^{3+} to the solutions, but the percentage of copper extraction increased by adding Fe^{2+} . After 14 days of leaching time, 10%, 26%, and 40% of copper was leached from chalcopyrite with 0.1 mol/L Fe^{3+} , 0.05 mol/L $\text{Fe}^{3+} + 0.05$ mol/L Fe^{2+} , and 0.1 mol/L Fe^{2+} , respectively. Moreover, leaching of chalcopyrite in Fe^{3+} solutions was lower than that in blank solutions. The initial mixed potentials in three kinds of leaching systems were 510, 420, and 270 mV, respectively (Fig. 3.17b). In 0.1 mol/L Fe^{3+} , and 0.05 mol/L $\text{Fe}^{3+} + 0.05$ mol/L Fe^{2+} solutions, the mixed potential decreased with leaching time owing to the precipitation of Fe^{3+} (Eq. 3.43) and/or reduction Fe^{3+} to Fe^{2+} (Eq. 3.48) (Qin et al., 2013). However, in 0.1 mol/L Fe^{2+} solutions, the mixed potential increased from 270 to 340 mV in the first 3 days, and then it slowly increased from 340 to 370 mV. Although the mixed potential was less than 340 mV in first 3 days, 11% of copper was leached from chalcopyrite. It was higher than copper extracted from chalcopyrite in 0.1-mol/L Fe^{3+} solutions after 14 days. Therefore, there was a clear benefit in leaching chalcopyrite within the low mixed potential or ratio of Fe^{3+} to Fe^{2+} . The total iron concentrations in leaching solution are shown in Fig. 3.17C. During leaching, precipitation of Fe^{3+} as shown in Eq. (3.48) caused a decrease in the total iron concentration:



The Raman spectra of CuFeS_2 , S^0 , and jarosite shown in Fig. 3.18 are used as a reference. Figs. 3.19 and 3.20 show XRD and Raman patterns of chalcopyrite leached after 14 days. The Raman spectrum of the original CuFeS_2 indicates an intense band at 290 cm^{-1} and additional low-intensity bands at 319, 351, and 375 cm^{-1} ; the Raman spectrum for S^0 has three bands at 153, 219, and 472 cm^{-1} ; jarosite has five intense bands at 222, 433, 624, 1006, and 1100 cm^{-1} , and three additional low-intensity bands at 298, 572, and 1151 cm^{-1} , respectively.

Smaller amounts of elemental sulfur were detected by XRD when chalcopyrite was leached in solutions at pH 1.6 (Fig. 3.19A); the same result was obtained by Raman analysis (Fig. 3.20A). The main leaching product of chalcopyrite leached with 0.1 mol/L Fe^{2+} and 0.05 mol/L $\text{Fe}^{2+} + 0.05$ mol/L Fe^{3+} was elemental sulfur and jarosite (Fig. 3.19B–C), but leached with 0.1 mol/L Fe^{3+} , the main leaching product was jarosite, together with lesser amounts of elemental sulfur (Fig. 3.19D). Raman analysis indicated that a significant amount of elemental sulfur and jarosite covered the chalcopyrite surface after chalcopyrite leaching with 0.1 mol/L Fe^{2+} (Fig. 3.20B). Only a low-intensity band of chalcopyrite was detected at 288 cm^{-1} . When chalcopyrite was leached with 0.1 mol/L Fe^{2+} or 0.05 mol/L $\text{Fe}^{2+} + 0.05$ mol/L Fe^{3+} , except for chalcopyrite bands centered at about 290, 317, and 350 cm^{-1} , only bands of jarosite were detected on the chalcopyrite surface (Fig. 3.20C and D).

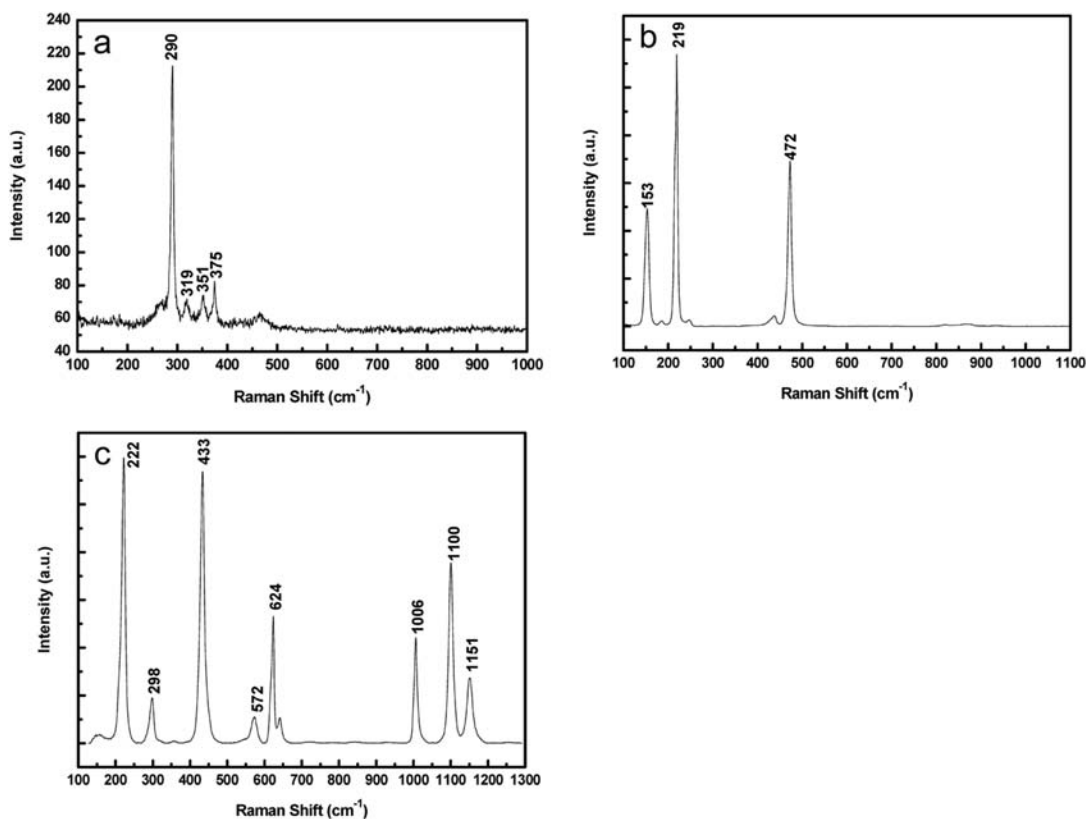


FIGURE 3.18 Raman spectra of CuFeS₂ (A), S⁰ (B), and jarosite (C). From Yang, C., Qin, W., Zhao, H., Wang, J., & Wang, X. (2018). Mixed potential plays a key role in leaching of chalcopyrite: Experimental and theoretical analysis. *Industrial and Engineering Chemistry Research*, 57(5), 1733–1744. <https://doi.org/10.1021/acs.iecr.7b02051>.

Jarosite was detected by XRD and/or Raman spectroscopy during the leaching of chalcopyrite. However, D'Hugues et al. (2002) and Gu et al. (2013) found no evidence of jarosite hindering chalcopyrite chemical or bioleaching. Qin et al. (2013) reported that 92.5% Cu was extracted from chalcopyrite by moderately thermophilic microorganisms although a significant amount of S⁰ and jarosite formed on the surface of chalcopyrite; similar results were obtained by Yang et al. (2013, 2015, 2016). According to our viewpoint, elemental sulfur and jarosite were not candidates for a passivated chalcopyrite surface.

The leaching results show that leaching of chalcopyrite was accelerated by Fe²⁺. This was similar to a previous study suggesting that at low mixed potential, chalcopyrite was first reduced to chalcocite; then, the formed chalcocite was oxidized by Fe³⁺ (Hiroyoshi, Hirota, Hirajima, & Tsunekawa, 1997; Hiroyoshi, Kuroiwa, Miki, Tsunekawa, & Hirajima, 2004; Hiroyoshi, Miki, Hirajima, & Tsunekawa, 2000, 2001; Zhao et al., 2015). Chalcopyrite was reduced to bornite and/or chalcocite in the cathodic peak (potential less than 100 mV versus SHE) according to Eqs. (3.49)–(3.51), and then chalcocite was oxidized in the anodic peak: Cu₂S → Cu_{1.92}S → Cu_{1.60}S → CuS (Gu, Hu, Zhang, Xiong, & Yang, 2013; Qin et al., 2013).

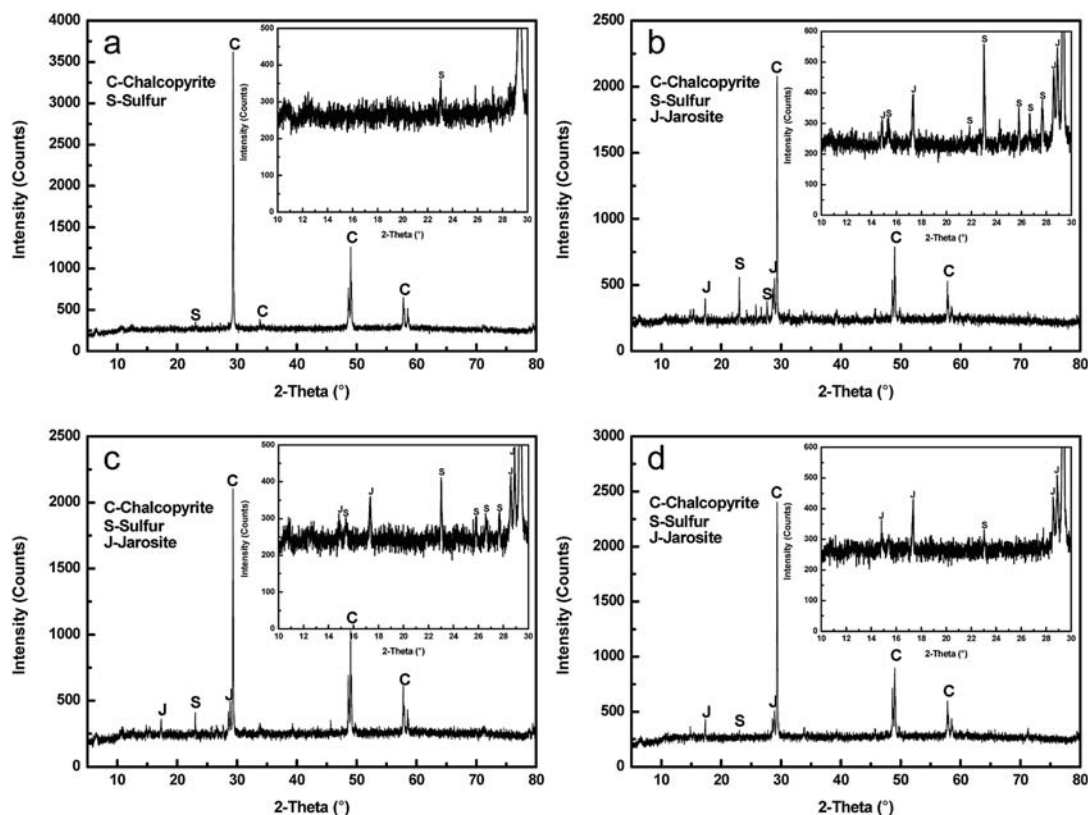
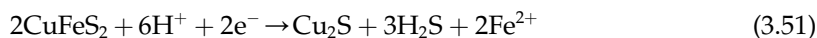
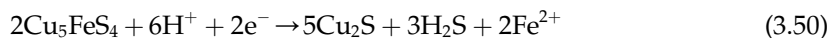
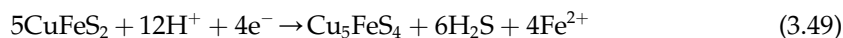


FIGURE 3.19 XRD patterns of chalcopyrite leached in solutions, pH 1.6, after 14 days: (A) blank, (B) 0.1 mol/L FeSO_4 , (C) 0.05 mol/L FeSO_4 + 0.025 mol/L $\text{Fe}_2(\text{SO}_4)_3$, and (D) 0.05 mol/L $\text{Fe}_2(\text{SO}_4)_3$. From Yang, C., Qin, W., Zhao, H., Wang, J., & Wang, X. (2018). Mixed potential Plays a key role in leaching of chalcopyrite: Experimental and theoretical analysis. *Industrial and Engineering Chemistry Research*, 57(5), 1733–1744. <https://doi.org/10.1021/acs.iecr.7b02051>.

Bornite and/or chalcocite was detected by Liu et al. (2015a, 2015b) when the mixed potential was less than 500 mV versus SCE; the covellite was detected when mixed potential was at about 550 mV versus SCE. The covellite was also detected by Yang et al. (2015) when bio-leaching chalcopyrite with different $\text{Fe}^{2+}/\text{Fe}^{3+}$ ratios at 30 and 48°C. The chalcocite was not detected by XRD and Raman in our experiments because the chalcocite was easily oxidized by Fe^{3+} :



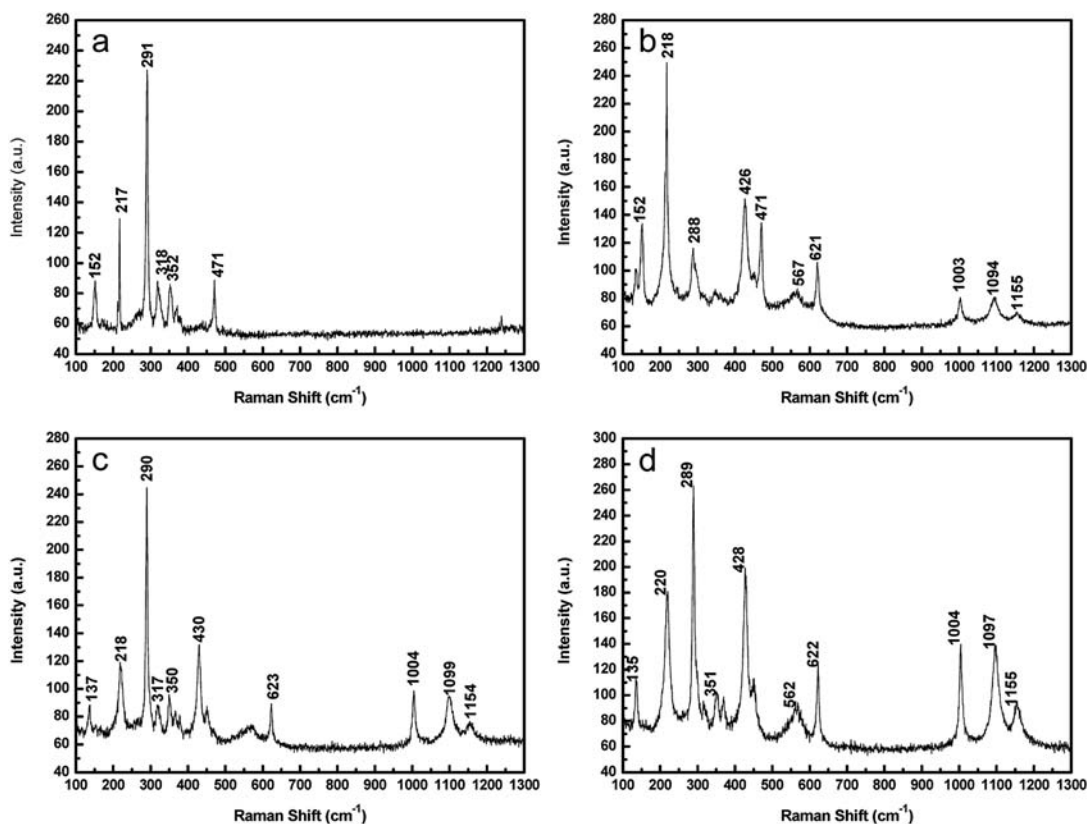


FIGURE 3.20 Raman patterns of chalcopyrite leached in solutions, pH 1.6, after 14 days: (A) blank, (B) 0.1 mol/L FeSO_4 , (C) 0.05 mol/L FeSO_4 + 0.025 mol/L $\text{Fe}_2(\text{SO}_4)_3$, and (D) 0.05 mol/L $\text{Fe}_2(\text{SO}_4)_3$. From Yang, C., Qin, W., Zhao, H., Wang, J., & Wang, X. (2018). Mixed potential plays a key role in leaching of chalcopyrite: Experimental and theoretical analysis. *Industrial and Engineering Chemistry Research*, 57(5), 1733–1744. <https://doi.org/10.1021/acs.iecr.7b02051>.

3.4.2 Effect of Cu^{2+} ions on chalcopyrite leaching

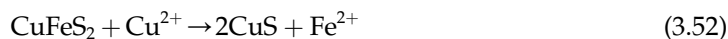
Two grams of chalcopyrite powder (100% passing -0.074 mm) and 100 mL of sulfuric acid solution (pH 1.6) was added to 250-mL flasks, and specific metal ion (Cu^{2+} , Fe^{2+} , and Fe^{3+}) concentrations were added to the solution (Table 3.4). The leaching experiments were conducted at 50°C at 160 rpm. Water lost by evaporation was supplemented periodically with deionized water until the mass of the flask equaled its initial mass. The solution pH was adjusted periodically to 1.6 with diluted sulfuric acid. Concentrations of Cu and Fe in solution were analyzed by ICP–AES. The solution pH was measured with a pH meter (PHSJ-4A) and solution potentials were measured with a saturated Ag/AgCl reference electrode and a Pt electrode. After leaching, the leaching residuals were washed with sulfuric acid solutions, pH 1.6, and dried in a vacuum box at 50°C . The solid samples were examined by Raman spectroscopy with a 633-nm He–Ne laser (Horiba Jobin Yvon Labram HR800). The chemical equilibrium in solution was calculated using Visual MINTEQ 3.0 at 50°C . The redox couples of $\text{Cu}^+/\text{Cu}^{2+}$, $\text{Fe}^{2+}/\text{Fe}^{3+}$, and $\text{HS}^-/\text{SO}_4^{2-}$ were chosen.

TABLE 3.4 Leaching experiments setup.

No.	CuFeS ₂ (g)	Solution (mL)	Cu ²⁺ (mol/L)	Fe ²⁺ (mol/L)	Fe ³⁺ (mol/L)
1	2	100	0	0	0
2	2	100	0.02	0	0
3	2	100	0.05	0	0
4	2	100	0.1	0	0
5	2	100	0.1	0.1	0
6	2	100	0.1	0.05	0.05
7	2	100	0.1	0	0.1

Data from Yang, C., Jiao, F., & Qin, W. (2018). Leaching of chalcopyrite: An emphasis on effect of copper and iron ions. *Journal of Central South University*, 25, 2380–2386. <https://doi.org/10.1007/s11771-018-3922-5>.

Fig. 3.21 shows the change in total copper concentration, total iron concentration, and redox potential during the leaching of chalcopyrite with different Cu²⁺ concentrations. Fig. 3.21A indicates that when the added Cu²⁺ concentration was no more than 0.02 mol/L, the copper concentration in solution increased with time. However, when 0.05 and 0.1 mol/L Cu²⁺ was added to the solution, the concentration of copper was less than the initial value during most of the leaching time, with the precipitation of the Cu²⁺ as copper compounds. The Fe concentration increased with an increase in time and reached 0.3 g/L after 14 days of leaching (Fig. 3.21B). During the whole leaching process, the redox potential was maintained at about 560 mV (Fig. 3.21C). The same experimental phenomenon was observed by Zies et al. (1916). The results of the solution chemistry calculation indicated that the chalcopyrite could transform into covellite by substituting Fe²⁺ with Cu²⁺, as shown in Eq. (3.52) (Fig. 3.21D):



Raman spectra of the original CuFeS₂ and leaching residuals are shown in Fig. 3.22. The original CuFeS₂ had an intense band at 290 cm⁻¹ and three low-intensity bands at 319, 351, and 375 cm⁻¹, which are in agreement with the observations of Majuste et al. (2012). When chalcopyrite was leached in a blank solution, three additional bands were detected at 152, 217, and 471 cm⁻¹; these bands can be assigned to elemental sulfur (153, 219, and 472 cm⁻¹). The additional band detected at 468 cm⁻¹ after chalcopyrite leaching with 0.1 mol/L Cu²⁺ can be assigned to CuS.

Fig. 3.23A shows that copper extraction increased with a decrease in Fe³⁺ concentration. In 0.1 mol/L Cu²⁺ + 0.1 mol/L Fe²⁺ and 0.1 mol/L Cu²⁺ + 0.05 mol/L Fe²⁺ + 0.05 mol/L Fe³⁺ solutions, the leaching behaviors of Cu and Fe were similar. The concentrations of copper in solution were less than their initial value after 1 day of leaching, and then the copper extraction increased with time. The total iron concentration increased owing to the dissolution of chalcopyrite, whereas it decreased owing to the precipitation of Fe³⁺, as shown in Eq. (3.53) (Fig. 3.23B) (Wu et al., 2015; Yang et al., 2011). In the 0.1-mol/L Cu²⁺ + 0.1-mol/L Fe²⁺ leaching system, oxidation of Fe²⁺ to Fe³⁺ by dissolved oxygen caused an increase

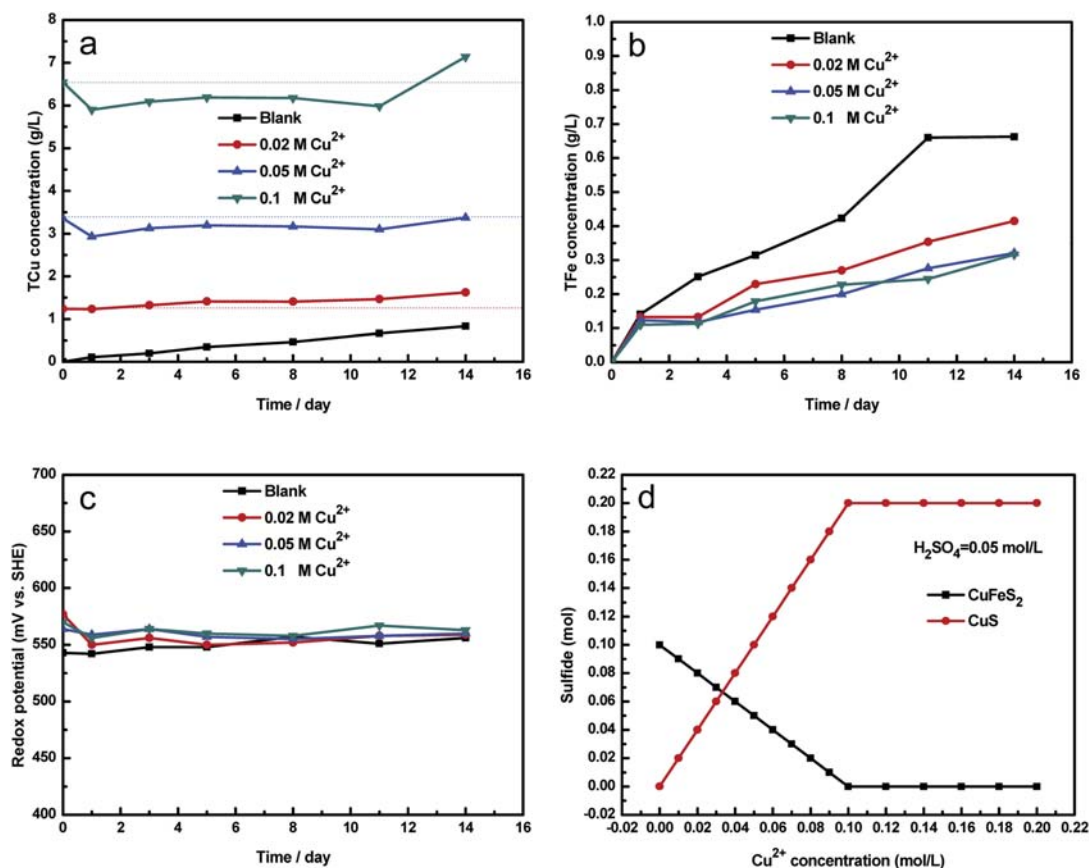
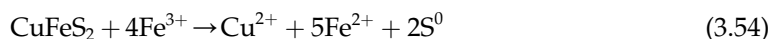
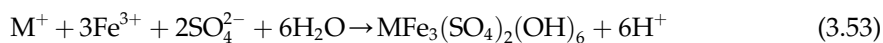


FIGURE 3.21 Effect of Cu^{2+} ions on leaching of chalcopyrite: (A) concentration of copper, (B) concentration of total dissolved iron, (C) redox potentials, (D) sulfide compositions changed with Cu^{2+} concentration (calculation conditions: 0.1 mol of chalcopyrite in 1-L solution). From Yang, C., Jiao, F., & Qin, W. (2018). Leaching of chalcopyrite: An emphasis on effect of copper and iron ions. Journal of Central South University, 25, 2380–2386. <https://doi.org/10.1007/s11771-018-3922-5>.

in redox potential from 520 to 580 mV in first 3 days. However, in 0.1-mol/L Cu^{2+} + 0.05-mol/L Fe^{2+} + 0.05-mol/L Fe^{3+} solutions, the redox potential decreased from 640 to 590 mV owing to precipitation of the Fe^{3+} (Eq. 3.53) and/or reduction of Fe^{3+} to Fe^{2+} (Eq. 3.54). In 0.1-mol/L Cu^{2+} + 0.1-mol/L Fe^{3+} solutions, the concentrations of copper in solution were almost maintained at their initial value during the whole leaching process. The total iron concentration decreased from 4.8 to 3.5 g/L owing to the precipitation of Fe^{3+} , as shown in Eq. (3.53). The redox potential decreased from 730 to 590 mV owing to the precipitation of Fe^{3+} and/or reduction of Fe^{3+} to Fe^{2+} (Qin et al., 2013):



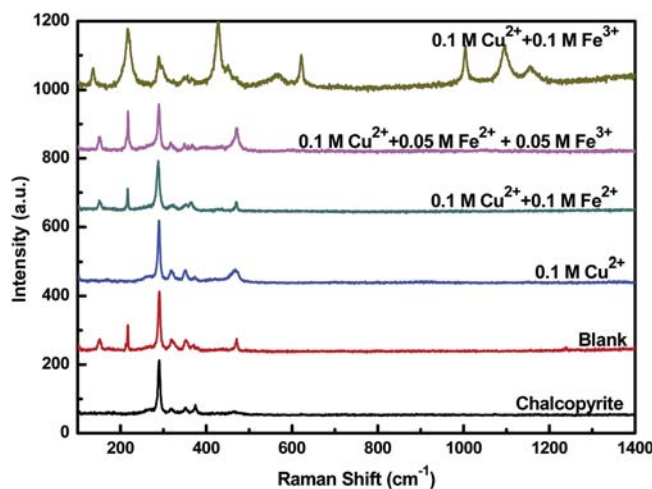


FIGURE 3.22 Raman patterns of chalcopyrite and leaching residuals. From Yang, C., Jiao, F., & Qin, W. (2018). *Leaching of chalcopyrite: An emphasis on effect of copper and iron ions*. Journal of Central South University, 25, 2380–2386. <https://doi.org/10.1007/s11771-018-3922-5>.

Similar to the blank (Fig. 3.22), element sulfur was detected by Raman spectroscopy when chalcopyrite was leached with 0.1 mol/L Cu^{2+} + 0.1 mol/L Fe^{2+} and 0.1 mol/L Cu^{2+} + 0.05 mol/L Fe^{2+} + 0.05 mol/L (Fig. 3.22). Jarosite, which has five intense bands at 222, 433, 624, 1006, and 1100 cm^{-1} and three low-intensity bands at 298, 572, 1151 cm^{-1} , respectively, was detected when chalcopyrite was leached in a 0.1-mol/L Cu^{2+} + 0.1-mol/L Fe^{3+} solution (Fig. 3.22).

Fig. 3.24A shows that the redox potential is proportional to the $\log(\text{Fe}^{3+}/\text{Fe}^{2+})$ and independent of the Cu^{2+} . Therefore, the redox potential can be used to indicate the change in Fe^{3+} and Fe^{2+} . The impact of the ratio of $\text{Fe}^{3+}/\text{Fe}^{2+}$ on sulfide compositions in the solution is shown in Fig. 3.24B. Compared with Fig. 3.21D, chalcocite was formed when Fe^{3+} and Fe^{2+} were added to the solution. Furthermore, chalcopyrite, covellite, and chalcocite coexisted in the solution when the concentration of Fe^{2+} was higher than 8.00×10^{-2} mol/L (or concentration of Fe^{3+} was less than 2.00×10^{-2} mol/L). When the concentration of Fe^{2+} decreased from 9.99×10^{-2} to 8.00×10^{-2} mol/L, the content of chalcopyrite and chalcocite decreased from 6.73×10^{-3} to 0 mol and 8.99×10^{-3} to 3.33×10^{-3} mol, respectively, but the content of covellite increased from 1.75×10^{-1} to 1.93×10^{-1} mol. Then, the content of covellite decreased from 1.93×10^{-1} to 1.67×10^{-1} mol whereas the content of chalcocite increased from 3.33×10^{-3} to 1.67×10^{-2} mol with a decrease in concentration of Fe^{2+} from 8.00×10^{-2} to 1.00×10^{-4} mol/L. The dissolution of sulfur increased from 2.27×10^{-3} to 1.67×10^{-2} mol with a decrease in concentration of Fe^{2+} from 9.99×10^{-2} to 1.00×10^{-4} mol/L (Fig. 3.24c). The balance redox potential in solution is shown in Fig. 3.24D.

The results of the leaching experiment showed that copper extraction decreased with an increase in Fe^{3+} concentration, which is in agreement with the previous study (Qin et al., 2013; Yang, Liu, & Chen, 2015; Zhao et al., 2015). Hiroyoshi et al. (1997, 2000, 2001, 2004) suggested that chalcopyrite was first reduced to chalcocite when Fe^{2+} ions and lesser amounts of Cu^{2+} ions were present in the leaching solution, and then the formed chalcocite was oxidized

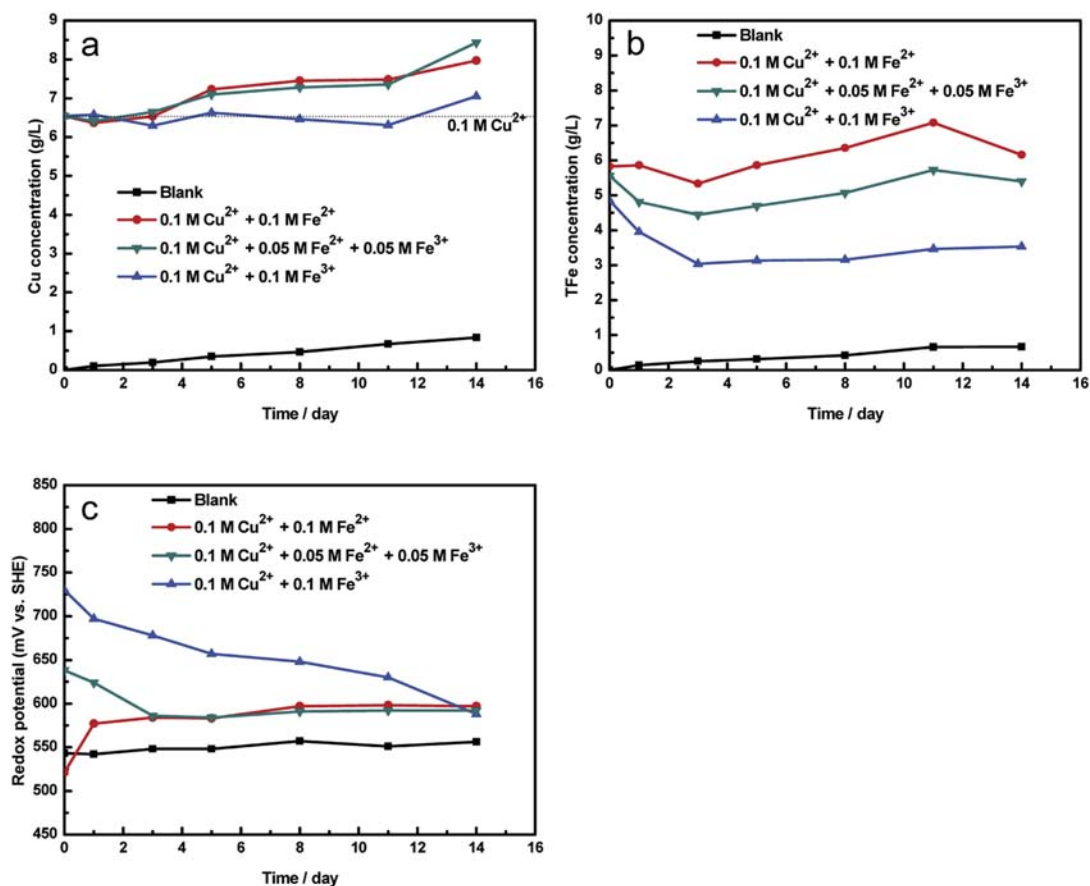


FIGURE 3.23 Effect of Cu²⁺, Fe²⁺, and Fe³⁺ ions on leaching of chalcopyrite: (A) concentrations of copper, (B) concentrations of total dissolved iron, and (C) redox potentials. From Yang, C., Jiao, F., & Qin, W. (2018). Leaching of chalcopyrite: An emphasis on effect of copper and iron ions. *Journal of Central South University*, 25, 2380–2386. <https://doi.org/10.1007/s11771-018-3922-5>.

by Fe³⁺. Fig. 3.24B shows that chalcopyrite, covellite, and chalcocite coexisted in the solution when the concentration of Fe²⁺ was higher than 8.00×10^{-2} mol/L and the concentration of Fe³⁺ was less than 2.00×10^{-2} mol/L (redox potential < 630 mV). Fig. 3.24C also shows that the dissolution of sulfur increased with an increase in Fe³⁺ concentration. However, during the leaching of chalcopyrite with Fe³⁺ or a high redox potential solution, less Cu was extracted owing to the formation of passive films consisting of disulfide and polysulfide (Wu et al., 2015).

Cu²⁺ replaced Fe in the chalcopyrite lattice and was converted into covellite; thus, a decrease in copper concentration was observed. Fe²⁺ and Fe³⁺ had a key role in forming chalcocite during the leaching of chalcopyrite with Cu²⁺. Fe²⁺ ions significantly accelerated the leaching of chalcopyrite.

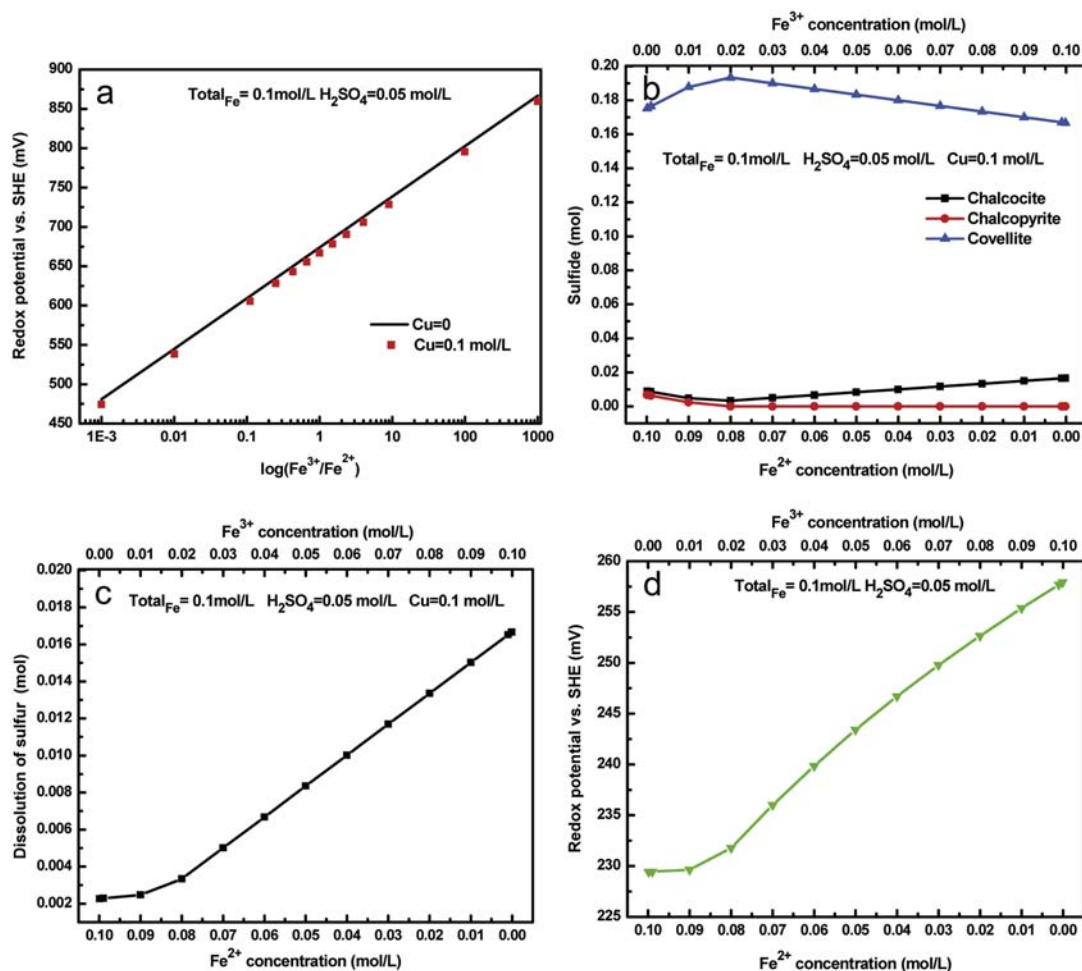
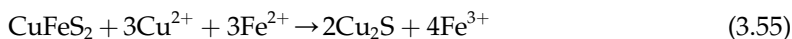
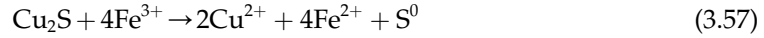
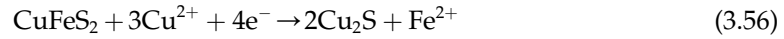


FIGURE 3.24 Relationship between the redox potential and ratio of $\text{Fe}^{3+}/\text{Fe}^{2+}$: (A) The sulfide compositions, (B) dissolution of sulfur, and (C) balance redox potential (D) changed with the Fe^{2+} and Fe^{3+} concentration (calculation conditions: 0.1 mol of chalcopyrite in 1-L solution). From Yang, C., Jiao, F., & Qin, W. (2018). *Leaching of chalcopyrite: An emphasis on effect of copper and iron ions*. Journal of Central South University, 25, 2380–2386. <https://doi.org/10.1007/s11771-018-3922-5>.

3.4.3 Thermodynamic calculation

Many researchers proposed that chalcopyrite can be reduced to chalcocite (Cu_2S) at a low redox potential, and the formed chalcocite (Cu_2S) can be rapidly oxidized (Elsherief, 2002; Gu, Hu, Zhang, Xiong, & Yang, 2013; Hiroyoshi, Miki, Hirajima, & Tsunekawa, 2000, 2001; Third, Cord-Ruwisch, & Watling, 2002). The process can be interpreted by chemical reactions as Eqs. (3.55)–(3.58):





These chemical reactions are mainly determined by redox potentials, concentrations of ferric ions, ferrous ions, and cupric ions. These factors can be controlled to enhance these chemical reactions, thus accelerating the kinetics of chalcopyrite dissolution.

For the reaction of Eq. (3.55), the change in Gibbs free energy can be given by Eq. (3.59):

$$\Delta G = \Delta G^0 + RT \ln \frac{(a\text{Fe}^{3+})^4}{(a\text{Fe}^{2+})^3 (a\text{Cu}^{2+})^3} = -nEF \quad (3.59)$$

where ΔG is the Gibbs free energy, R is the gas constant (8.314 J/molK), T is the absolute temperature (318 K), F is the Faraday constant (96,485 C/mol), n is the number of the charge transfer., and E is the potential of the reaction.

It was assumed that the redox potential of leaching solution was mainly determined by the Nernst equation in Eq. (3.60):

$$E = E^0 + \frac{RT}{F} \ln \frac{[\text{Fe}^{3+}]}{[\text{Fe}^{2+}]} \quad (3.60)$$

Based on these equations, the maximum redox potential for the reaction in Eq. (3.55) can be defined as a high critical potential (E_{H1}) which can be calculated as Eq. (3.61):

$$E_{H1} = 482 + 47.3 \lg(a\text{Cu}^{2+}) - 15.77 \lg(a\text{Fe}^{2+}) / \text{mV} \quad (3.61)$$

Similarly, the change in Gibbs free energy in Eq. (3.56) can be given by Eq. (3.62):

$$\Delta G = \Delta G^0 + RT \ln \frac{(a\text{Fe}^{2+})}{(a\text{Cu}^{2+})^3} = -nEF \quad (3.62)$$

Accompanied by the Nernst equation in Eq. (3.59), the critical redox potential (E_{H2}) can be calculated as shown in Eq. (3.63):

$$E_{H2} = 481 + 47.3 \lg(a\text{Cu}^{2+}) - 15.77 \lg(a\text{Fe}^{2+}) / \text{mV} \quad (3.63)$$

It clearly shows that the critical redox potential of Eq. (3.55) is the same as that of Eq. (3.56). The high critical redox potential (E_H) of chalcopyrite leaching was a function of the concentrations of Cu^{2+} and Fe^{2+} , indicating that the value of E_H mainly depends on the concentrations of Cu^{2+} and Fe^{2+} . Chalcopyrite was able to be reduced to chalcocite (Cu_2S) in the

presence of Cu^{2+} and Fe^{2+} when the solution potential was lower than the value of E_H , and then the dissolution of chalcopyrite can be accelerated.

In addition, the further oxidation of formed chalcocite (Cu_2S) (Eq. 3.57) can be described by Eq. (3.64):

$$\Delta G = \Delta G^0 + RT \ln \frac{(a\text{Fe}^{2+})^4 (a\text{Cu}^{2+})^2}{(a\text{Fe}^{3+})^4} = -nEF \quad (3.64)$$

The low critical redox potential (E_{L1}) can be calculated as shown in Eq. (3.65):

$$E_{L1} = 362 + 31.5 \lg(a\text{Cu}^{2+})/\text{mV} \quad (3.65)$$

Similarly, the change in Gibbs free energy in Eq. (3.58) can be given by Eq. (3.66):

$$\Delta G = \Delta G^0 + RT \ln(a\text{Cu}^{2+})^4 = -nEF \quad (3.66)$$

The critical redox potential (E_{L2}) can be calculated as in Eq. (3.67):

$$E_{L2} = 401 + 29.5 \lg(a\text{Cu}^{2+})/\text{mV} \quad (3.67)$$

The low critical redox potential (E_L) was a function of concentrations of Cu^{2+} , indicating that the critical redox potential of oxidation of chalcocite (Cu_2S) mainly depended on the concentrations of Cu^{2+} . In addition, the coefficients of equations of critical potential were mainly determined by the temperature of solution (T), but the values of their intercepts were not affected by the temperature. Therefore, chalcopyrite can be reduced to chalcocite (Cu_2S) in the optimum range of redox potential ($E_L \sim E_H$), thus resulting in the acceleration of chalcopyrite dissolution.

Actually, redox potential is always higher than the low optimum redox potential (E_L) in both bioleaching and chemical leaching. Therefore, the high critical redox potential (E_H) is the most important point for accelerating the dissolution of chalcopyrite in bioleaching or chemical leaching.

3.4.4 Impact of electronic structure on leaching of chalcopyrite

To understand the leaching of chalcopyrite better, the band theory was used to analyze the oxidation and reduction of chalcopyrite. The bandgap of chalcopyrite was in the range of 0.53–0.6 eV (Li et al., 2013), and the Fermi energy was calculated to be -5.4 eV (Chen et al., 2013). The valence band consisted of S 3p and Cu 3d together with less Fe 3d, and the conduction band consisted of Fe 3d together with less S 3p (Li et al., 2013). The initial oxidation of chalcopyrite should be related to the electrons in occupied states near Fermi energy; the initial reduction of chalcopyrite should be related to the unoccupied states near Fermi energy. Therefore, empty states of Fe 3d captured electrons from filled states of reducing agents during the reduction of chalcopyrite; but unoccupied states of oxidizing agent captured electrons from occupied states of S 3p and Cu 3d during the oxidation of

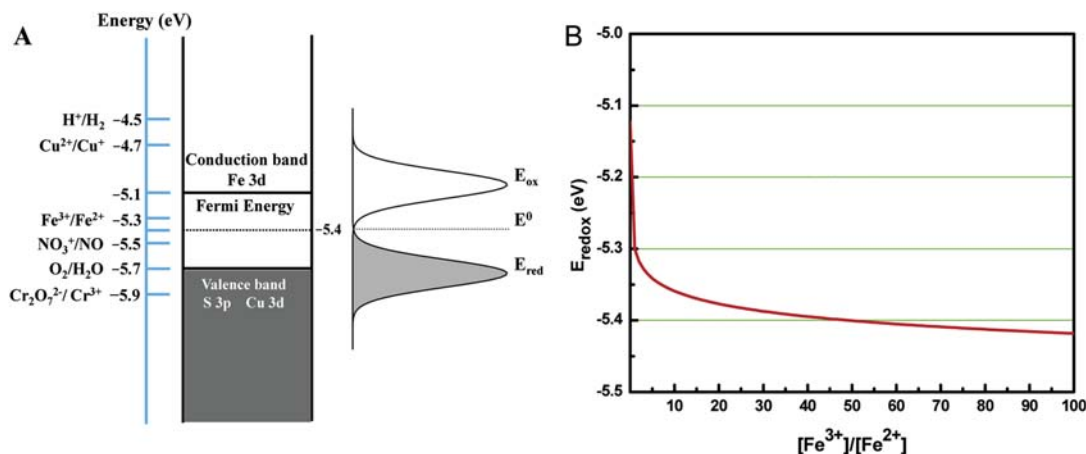


FIGURE 3.25 Schematic illustration for band structure of chalcopyrite (A), and relation between E_{redox} and ratio of Fe^{3+}/Fe^{2+} (B). From Yang, C., Qin, W., Zhao, H., Wang, J., & Wang, X. (2018). Mixed potential plays a key role in leaching of chalcopyrite: Experimental and theoretical analysis. *Industrial and Engineering Chemistry Research*, 57(5), 1733–1744. <https://doi.org/10.1021/acs.iecr.7b02051>.

chalcopyrite. The energy median between the occupied and unoccupied states (E_{redox}) is related directly to the oxidation potential of the redox couple (Morrison, 1978), and the E_{redox} of redox couple of H^+/H_2 was -4.5 eV (Bard & Faulkner, 2001; Crundwell, 1988). Thus, the E_{redox} of Cu^{2+}/Cu^+ , Fe^{3+}/Fe^{2+} , NO_3^-/NO , O_2/H_2O , $Cr_2O_7^{2-}/Cr^{3+}$ was calculated to be -4.7 , -5.3 , -5.5 , -5.7 , and -5.9 eV, respectively (Bard & Faulkner, 2001). Electron transfer can take place from any occupied state that is matched in energy with an unoccupied state.

Fig. 3.25A shows that the E_{redox} of Cu^{2+}/Cu^+ was higher than the bottom of the conduction band; the empty states of chalcopyrite overlap with the filled states of Cu^+ . Therefore, chalcopyrite captured electrons from Cu^+ and was reduced. Zies et al. (1916) proved that chalcopyrite converted into chalcocite was faster in the presence of Cu^+ than in the presence of Cu^{2+} . The E_{redox} of Fe^{3+}/Fe^{2+} was located in the bandgap and was higher than the Fermi energy of chalcopyrite, although increasing the ratio of Fe^{3+} to Fe^{2+} would cause E_{redox} to be lower than the Fermi level, but still higher than the top of the valence band (Fig. 3.25). Thus, Fe^{3+} is difficult to capture electrons from the surface of chalcopyrite; chalcopyrite is difficult to be leached by Fe^{3+} . When chalcopyrite was leached with 1 mol/L H_2SO_4 and 1 mol/L $Fe_2(SO_4)_3$ at $50^\circ C$, only 3% of Cu was extracted after 24 h. Similarly, in 0.1-mol/L Fe^{3+} solutions at pH 1.6, the percentage of copper extraction was only 10% after 14 days. Fig. 3.25B also shows that decreasing the ratio of Fe^{3+} to Fe^{2+} would result in the E_{redox} being close to the bottom of the conduction band. Even the empty states of chalcopyrite overlap with filled states of Fe^{2+} , which correspond to a strongly enabled reduction of chalcopyrite and oxidation of Fe^{2+} . Many researchers suggested that chalcopyrite was reduced to chalcocite at low mixed potential or a ratio of Fe^{3+} to Fe^{2+} . The chalcocite was easily oxidized by Fe^{3+} ; thus, a high percentage of copper extraction was reached (Gu, Hu, Zhang, Xiong, & Yang, 2013; Hiroyoshi, Hirota, Hirajima, & Tsunekawa, 1997; Hiroyoshi, Miki, Hirajima, & Tsunekawa, 2001; Liang et al., 2011; Liu et al., 2015a; Liu, Xia, & Nie, 2015b; Qin et al., 2013; Zhao et al., 2015). In 0.1-mol/L Fe^{2+} solutions at pH 1.6, the percentage of copper extraction

finally reached 40% after 14 days of leaching time. Furthermore, chalcopyrite was leached with 1 mol/L H_2SO_4 and 3 mol/L HNO_3 or 1 mol/L H_2SO_4 and 0.3 mol/L $\text{K}_2\text{Cr}_2\text{O}_7$ at 50°C , 83% and 96% Cu was extracted after 24 h, respectively. Fig. 3.25A shows that the E_{redox} of NO_3^-/NO was lower than the Fermi level; therefore, NO_3^- captured electrons from chalcopyrite and was reduced; chalcopyrite was oxidized by NO_3^- . The empty states of $\text{Cr}_2\text{O}_7^{2-}$ overlapped with filled states of chalcopyrite, which corresponded to the strongly enabled reduction of $\text{Cr}_2\text{O}_7^{2-}$ and oxidation of chalcopyrite. Therefore, a higher percentage of copper extraction was obtained. The E_{redox} of redox couple of $\text{O}_2/\text{H}_2\text{O}$ was always below the Fermi energy and close to the valence band, so it is easy for O_2 to capture electrons from chalcopyrite surface, causing a high oxidation rate of chalcopyrite. However, the content of dissolved O_2 is extremely low at normal pressure, which is why high pressure was necessary for oxygen-rich leaching.

The electronic structure has a key role in the oxidation or reduction of chalcopyrite. Empty states of the oxidizing agent overlap with the filled states of chalcopyrite, or the filled states of the reducing agent overlap with the empty states of chalcopyrite, which correspond to the strongly enabled oxidation and reduction of chalcopyrite; thus, higher copper extraction was obtained.

3.5 Enhancing bioleaching of chalcopyrite by controlling the solution potential

The chalcopyrite sample used in the test was obtained from Meizhou, Guangdong Province, China. The chemical analysis of the ore sample showed Cu 31.12%, Fe 25.50%, S 32.79%, Pb 1.93%, Zn 0.36%, MgO 0.10%, CaO 0.12%, and Al_2O_3 0.14%. XRD of the sample showed that the main mineral was chalcopyrite. The chalcopyrite samples used for flask leaching were ground and screened to -0.038 mm.

Moderately thermophilic bacteria *Sulfobacillus thermosulfidooxidans* and *Archaea ferroplasma* sp. were enrichment cultured from a leaching solution sample obtained from Inner Mongolia Province, China, in 250-mL shake flasks using an orbital incubator with a stirring speed of 160 rpm at 50°C . *Acidithiobacillus ferrooxidans* was obtained from the Key Laboratory of Biometallurgy of Ministry of Education, Central South University, Changsha, China. It was cultured in 250-mL shake flasks using an orbital incubator with a stirring speed of 160 rpm at 30°C . The medium used for cell cultivation consisted of 3.0 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.1 g/L KCl, 0.5 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g/L K_2HPO_4 , and 0.01 g/L $\text{Ca}(\text{NO}_3)_2$. The moderately thermophilic bacteria were supplemented with yeast extract 0.02%. All bacterial cultures were subcultured into basal salts medium supplemented with 2% ore powder as the energy source. The resulting mixed culture was used as inocula for all experiments.

For bioleaching in shake flasks, cells were inoculated into 250-mL flasks containing 100 mL sterilized culture medium and 2 g chalcopyrite. The flasks were placed in an orbital shaker at 160 rpm and 50°C (moderately thermophilic microorganisms) or 30°C (*A. ferrooxidans*). The initial cell concentration was 1.0×10^7 cells/mL. Parallel experiments without cells but with the same culture medium and chalcopyrite were run as sterile controls. Many sets at different target pH values were set up. The solution pH was adjusted periodically to the

target pH values with diluted sulfuric acid. Water lost by evaporation was supplemented periodically by adding sterile water until the mass of the flask equaled its initial mass. Bioleaching of chalcopyrite by moderately thermophilic microorganisms was supplemented with yeast extract 0.02%.

The mineralogical compositions of solid samples were examined by XRD (Rigaku Dmax-2000). Soluble Cu and Fe were determined by ICP-AES and the ferrous concentration was determined by standard potassium dichromate titration (Peng et al., 2006). The pH of the supernatant was measured with a pH meter (PHSJ-4A) and the redox potentials in the leaching solution were measured with a Pt electrode with reference to a saturated calomel electrode.

Leaching characteristics of chalcopyrite by different cells and sterile controls, in terms of changes in the percentage of Cu extraction and concentrations of total dissolved iron and redox potentials, are shown in Fig. 3.26.

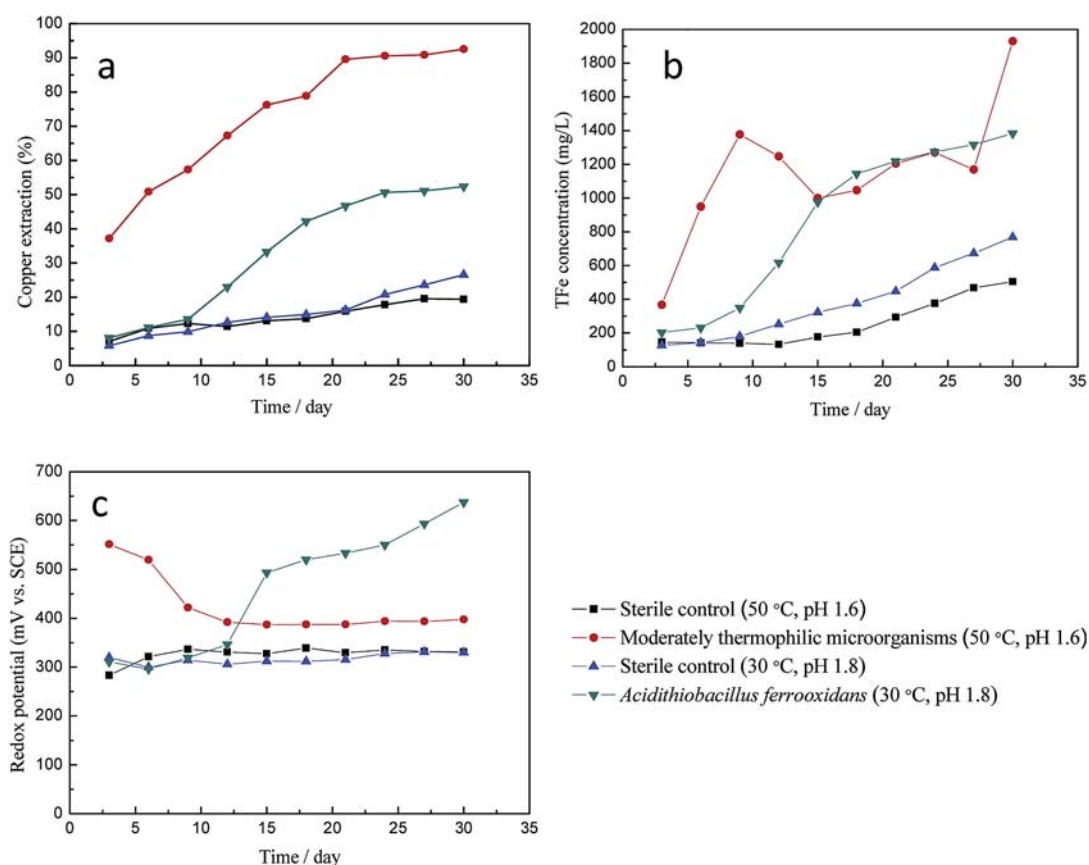
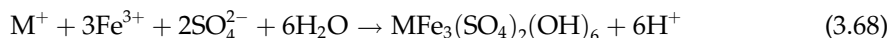


FIGURE 3.26 Copper extraction (A), concentrations of total dissolved iron (B), and redox potentials (C) in leach liquors during bioleaching of chalcopyrite. From Qin, W., Yang, C., Lai, S., Wang, J., Liu, K., & Zhang, B. (2013). Bioleaching of chalcopyrite by moderately thermophilic microorganisms. *Bioresource Technology*, 129, 200–208. <https://doi.org/10.1016/j.biortech.2012.11.050>.

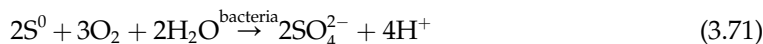
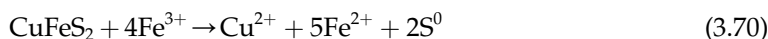
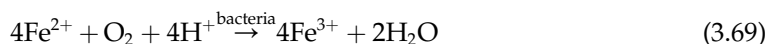
Fig. 3.26A shows that in the bioleaching and sterile controls leaching system, the percentage of Cu extraction increased with time and reached a maximum after 30 days. The final extraction percentage of Cu for leaching by moderately thermophilic microorganisms and sterile control at 50°C were 92.5% and 19.4%, respectively; in comparison, 52.4% Cu was bioleached by *A. ferrooxidans*, whereas 26.6% Cu was leached by sterile control at 30°C.

During the first 9 days of bioleaching by moderately thermophilic microorganisms, the concentrations of total dissolved iron increased to 1.4 g/L; then, they slightly decreased and stabilized at about 1.2 g/L owing to precipitation of the soluble ferric iron as jarosite (Eq. 3.68), and finally reached 1.9 g/L after 30 days of bioleaching (Fig. 3.26B). The concentrations of total dissolved iron steadily increased and reached 1.4 g/L, 0.5 g/L, and approximately 0.8 g/L after 30 days of leaching by *A. ferrooxidans* and sterile control at 50 and 30°C, respectively (Fig. 3.26B):



where M is a monovalent cation (e.g., H_3O^+ , Na^+ , K^+ , and NH_4^+).

In the first 3 days, the redox potentials of bioleaching with the moderately thermophilic microorganisms reached 550 mV because ferrous ions were oxidized to ferric by the microorganisms (Eq. 3.69), whereas the potential slowly decreased from 550 to 390 mV over the following 9 days because the rate of ferrous oxidation to ferric by the microorganisms was less than the consumption rate of ferric via chalcopyrite oxidation (Eqs. 3.70 and 3.71) or the precipitation of the soluble ferric iron as jarosite (Eq. 3.68), and then the solution potential was stabilized at about 400 mV. In contrast, the redox potentials steadily increased and reached 640 mV after 30 days of bioleaching by *A. ferrooxidans* while the solution potential of leaching by sterile control at 50 and 30°C was maintained at about 320 mV. Copper extraction continued until 92.5% of copper was recovered from chalcopyrite even though the solution potential was below 400 mV after 12 days of leaching by moderately thermophilic microorganisms. The copper extraction rate was superior to the rate obtained with the *A. ferrooxidans* mediated by a high-potential leach condition (above 400 mV after 12 days of leaching). Copper dissolution with *A. ferrooxidans* almost stopped when the solution potential reached 550 mV after 24 days of leaching:



There was a clear benefit in leaching chalcopyrite within the low solution potential compared with high-potential leaching. Nevertheless, the solution potential of the sterile control at 50 and 30°C was no more than 340 mV; 80.6% and 73.4% copper were still locked in the chalcopyrite. From these results, it was apparent that the solution potential of the bioleaching system needed to be controlled at an appropriate value (Hiroyoshi, Hirota, Hirajima, & Tsunekawa, 1997; Hiroyoshi, Miki, Hirajima, & Tsunekawa, 2001; Sandström, Shchukarev, & Paul, 2005; Vilcáez, Suto, & Inoue, 2008).

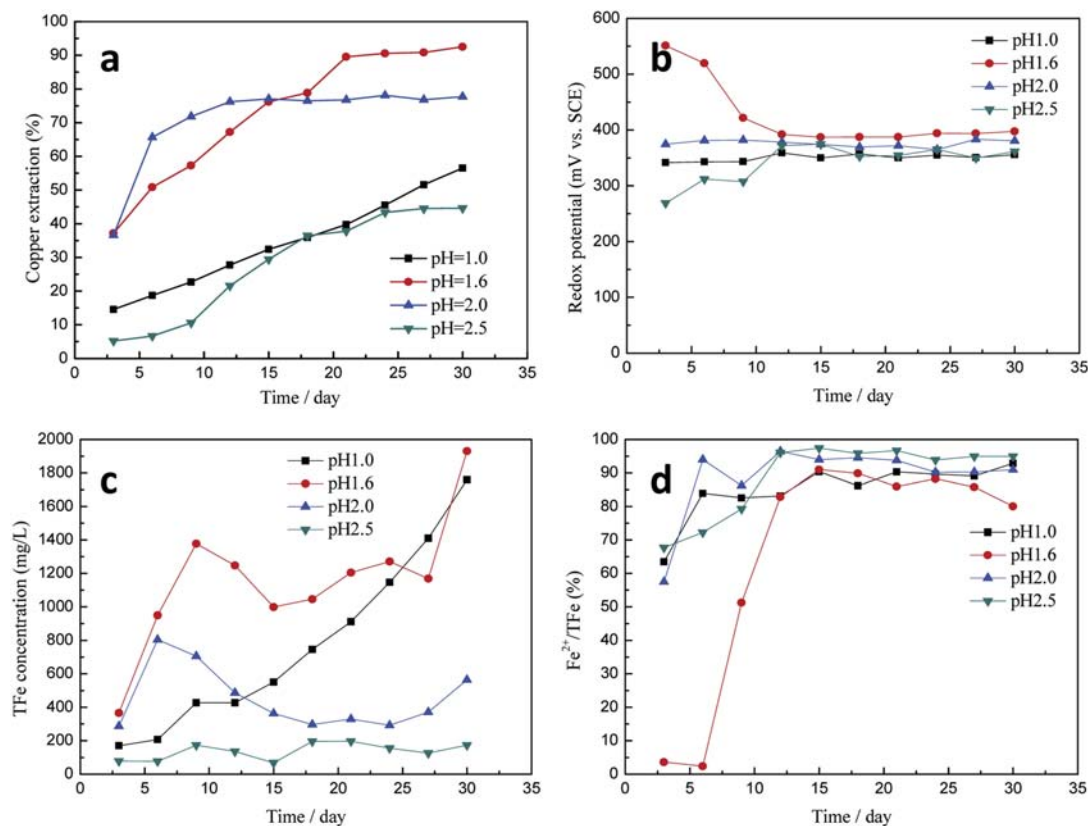
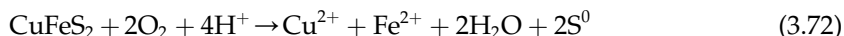


FIGURE 3.27 Copper extraction (A), redox potentials (B), concentrations of total dissolved iron, (C) ratio of Fe^{2+} to TFe, and (D) in leach liquors during the bioleaching of chalcopyrite by moderately thermophilic microorganisms at different pHs. From Qin, W., Yang, C., Lai, S., Wang, J., Liu, K., & Zhang, B. (2013). Bioleaching of chalcopyrite by moderately thermophilic microorganisms. *Bioresource Technology*, 129, 200–208. <https://doi.org/10.1016/j.biortech.2012.11.050>.

The leaching characteristics of chalcopyrite by cells at different pHs, in terms of changes in the percentage of Cu extraction, concentrations of total dissolved iron, ratio of Fe^{2+} to total Fe (TFe), and redox potentials are shown in Fig. 3.27. At pH 1.6, 92.5% Cu was bioleached after 30 days; in comparison, at pH 1.0 and 2.5, only 56.5% and 44.6% Cu were bioleached after 30 days, respectively; whereas at pH 2.0, 77.7% Cu was bioleached after 30 days (Fig. 3.27A).

Vilcáez and Inoue (2009) studied the effects of pH on chalcopyrite leaching and reported that a lower pH notably promoted the copper leaching rate (Eq. 3.72). Comparisons between copper extractions at different pH values (Fig. 3.27B) in bioleaching with moderately thermophilic microorganisms revealed that a highest Cu extraction percentage was obtained at pH 1.6, because moderately thermophilic microorganisms had better oxidation ferrous ions and/or elemental sulfur activity at pH 1.6 than others (pH 1.0, 2.0, and 2.5):



The high solution potential (above 400 mV) negatively reflected on copper recovery, achieving only 57.3% over 9 days at pH 1.6, compared with 71.8% with the low solution potential (about 380 mV) at pH 2.0. There was a great advantage (14.5% higher copper recovery) in leaching chalcopyrite within the low solution potential, compared with high potential leaching. At pH 1.0 and 2.5, the solution potential was no more than 360 mV, and 44% and 56% of copper were still locked in chalcopyrite. From the results, it was evident that the solution potential and pH needed to be controlled at appropriate values.

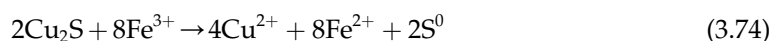
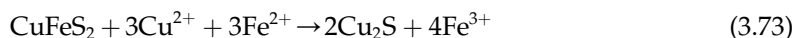
The concentrations of total dissolved iron steadily increased and reached 1.7 g/L after 30 days of bioleaching at pH 1.0 (Fig. 3.27C). At pH 1.6, during the first 9 days of bioleaching, the concentrations of total dissolved iron increased to 1.4 g/L and then slightly decreased and stabilized at about 1.2 g/L and finally reached 1.9 g/L after 30 days of bioleaching (Fig. 3.27C). The rational was to control the solution potential below 400 mV by precipitating the soluble ferric iron as jarosite. At pH 2.0, the total iron concentration increased to approximately 0.8 g/L after 6 days of bioleaching, but then it started to decrease (Fig. 3.27C) owing to jarosite precipitation. The entire time, the solution potential stabilized at about 380 mV. At pH 2.5, total iron remained below 200 mg/L (Fig. 3.27C) throughout the experiment because of brown iron (III) oxide precipitates on the surfaces of the ore material.

The most noteworthy observation from the results was that the copper dissolution stopped at pH 2.0 after 12 days. This phenomenon also reflected the concentrations of total dissolved iron, which started to decrease owing to ferric formation jarosite precipitation after 6 days. A second observation was the enhanced percentage of extraction obtained during the high total iron concentration (pH 1.6, more than 1.0 g/L), compared with the low total iron concentration (pH 2.0, lower than 0.5 g/L), although the solution potential was maintained at about 390 and 380 mV, respectively.

According to Eq. (3.70), increasing the concentration of the ferric ions in solution is supposed to result in higher amounts of copper release. However, dissolution of chalcopyrite would be favored when the redox potential is under a critical value, identified at approximately 310–400 mV (Ag/AgCl), or at an appropriate ratio of $\text{Fe}^{3+}/\text{Fe}^{2+}$ (Hiroyoshi, Miki, Hirajima, & Tsunekawa, 2001; Sandström, Shchukarev, & Paul, 2005; Vilcáez, Suto, & Inoue, 2008; Vilcáez, Yamada, & Inoue, 2009). Vilcáez et al. (2009) suggested that the leaching of chalcopyrite occurs through direct oxidation by ferric ion (Eq. 3.70), favored at high redox potential values (>450 mV versus Ag/AgCl), and indirectly through the formation of intermediates, favored at low redox potential values (<450 mV versus Ag/AgCl). Electrochemical studies have shown that chalcocite might be a possible intermediate compound during chalcopyrite oxidation, whether in acid or alkaline environments (Arce & González, 2002; Velásquez et al., 2005). Vilcáez and Inoue (2009) indicated that chalcopyrite dissolution is regarded as a cooperation process of cathode reduction and anode oxidation, which is controlled at the beginning by the concentrations of Fe^{2+} and Fe^{3+} , respectively. At high concentrations of Fe^{3+} , the rate of chalcopyrite oxidation tends to prevail over the rate of chalcopyrite reduction. On the contrary, at high concentrations of Fe^{2+} , the rate of chalcopyrite reduction may prevail over the rate of chalcopyrite oxidation. Therefore, it seems that at a low redox potential, the copper release rate is not controlled by the concentration of Fe^{3+} but by Fe^{2+} , which is extraordinarily important for the formation of Cu_2S (Vilcáez & Inoue, 2009). With increasing ratio of $\text{Fe}^{3+}/\text{Fe}^{2+}$, redox potential would rise continuously over a critical value for chalcocite formation, and only direct oxidation of chalcopyrite exists, which would be more insoluble, and would drag the copper releasing rate (Vilcáez & Inoue, 2009; Vilcáez, Suto, & Inoue, 2008). Hiroyoshi et al. (2001) reported that at a low redox potential,

chalcopyrite is reduced in the first step by ferrous ions to form chalcocite, which is more soluble at the cathode (Eq. 3.73), and the formed chalcocite is oxidized in the second step by ferric ions at the anode (Eq. 3.74).

Apparently, these reactions could happen synchronously only when a particular range of the redox potential is maintained (i.e., the redox potential must be low enough for chalcocite formation and high enough for the subsequent chalcocite oxidation). This model suggests that the leaching of chalcopyrite should be enhanced by increasing not only the ferric ion concentration but also the ferrous and cupric ion concentrations. In the absence of sufficient ferrous and cupric ions to accelerate the formation of chalcocite, the boosting effect of ferric ion appears to be attenuated or suppressed:



Owing to the relationship between the ferrous/ferric iron ratio and the iron-based solution potential, a higher total iron concentration would therefore contribute to a higher ferrous and ferric iron concentration when the solution potential is maintained at about 390 mV (Fig. 3.27D). Thus, when the solution potential is maintained at about 390 mV, increasing the concentration of total dissolved iron, which represents an accumulation of Fe^{3+} and Fe^{2+} , should favor chalcopyrite leaching.

XRD analysis indicated that a significant amount of elemental sulfur and jarosite was formed on the chalcopyrite surface after bioleaching (Fig. 3.28). The main leaching product

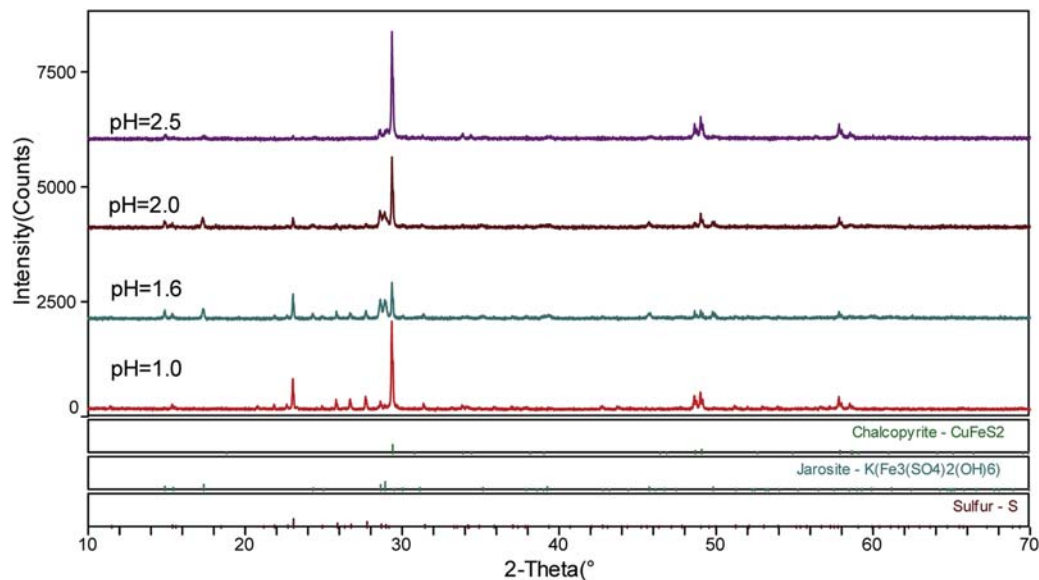


FIGURE 3.28 Mineralogical compositions of residuals in bioleaching experiment with moderately thermophilic microorganisms at different pHs. From Qin, W., Yang, C., Lai, S., Wang, J., Liu, K., & Zhang, B. (2013). Bioleaching of chalcopyrite by moderately thermophilic microorganisms. *Bioresource Technology*, 129, 200–208. <https://doi.org/10.1016/j.biortech.2012.11.050>.

of bioleaching at pH 1.0 was elemental sulfur, together with lesser amounts of jarosite, but at pH 2.0, the main leaching product was jarosite, together with lesser amounts of elemental sulfur. The leaching products of bioleaching at pH 1.6 were elemental sulfur and jarosite. When the solution pH was 2.5, only lesser amounts of jarosite were observed on the chalcopyrite surface. [Klauber \(2008\)](#) reported that the rate of jarosite formation increased dramatically with temperature, but the rate was slow below 60°C, and the rate was at a maximum at pH ~2. Jarosite formation was increasingly depressed at pH <1 or pH >3.

The copper-bearing sulfide ore used in column bioleaching was mixed with 4.5 kg–10 mm low-grade copper-bearing sulfide ore (Cu 0.51%) and 1.1 kg–3 mm high-grade copper-bearing sulfide ore. A chemical analysis of the artificial mixture of copper-bearing sulfide ore sample showed Cu 5.96%, Fe 14.06%, and S 8.1%. Mineralogical analysis of the ore sample showed that 53.36% and 45.97% Cu was present in primary copper sulfide and secondary copper sulfide, respectively. XRD analysis of the sample showed that the main copper minerals were chalcopyrite and bornite.

A representative artificial mixture of copper-bearing sulfide ore sample was loaded into a column (0.5 m tall, 15 cm in diameter) equipped with a 3-L solution reservoir, and a column leaching experiment was carried out at 50°C. Before inoculation, the charge was ideally preleached by a 2.5-L diluted sulfuric acid solution (the solution pH value was about 1.2) to control the discharged solution pH at a level for good bacterial activity. When the pH of the discharged solution was less than 2.0%, 5% (v/v) inoculums (moderately thermophilic microorganisms) with a cell density of about 10^7 cell/mL and nutrients of 3.0 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.5 g/L K_2HPO_4 , and 0.02% yeast extract were added to the discharged solution. The discharged solution pH value was adjusted periodically to about 1.2 during bioleaching.

[Fig. 3.29A](#) shows that in the column leaching, the percentage of Cu and Fe extraction increased with time, and a recovery of 55.3% Cu and 6.3% Fe was achieved in 70 days. The redox potential of the leaching solution was 280–450 mV ([Fig. 3.29B and C](#)). [Fig. 3.29D](#) shows the value of the leaching solution pH during column leaching.

Two regions can be identified where changes occur in the dissolution of copper-bearing sulfide ore. Bornite was dissolved in region I ([Fig. 3.29A](#)) and chalcopyrite was dissolved in region II ([Fig. 3.29A](#)). The copper dissolution rate in region I (48% of copper was leached in 43 days of bioleaching.) was higher than that obtained in region II (7% of copper was leached in 27 days of bioleaching.). This result showed that at low redox potential, bornite is dissolved in the first product. The analysis of CV results revealed that at low redox potential, the chalcopyrite is reduced to a series of intermediate products (such as talnakhite, bornite, and chalcocite) in the first product, and then the intermediate product (chalcocite) is oxidized. Therefore, the initial reduction of chalcopyrite to intermediate products is the rate-limiting step of chalcopyrite dissolution ([Liang et al., 2011](#)).

3.6 Summary

Fe dissolves preferentially from the chalcopyrite surface in the potential range of 475–700 mV, leaving behind an S_2^{2-} and S_n^{2-} passive film. The passive film consisting of S_2^{2-} and S_n^{2-} is oxidized when the applied potential is higher than 700 V, and transpassive dissolution of chalcopyrite occurs. Similar results were obtained by leaching chalcopyrite with $\text{Fe}_2(\text{SO}_4)_3$, HNO_3 , and $\text{K}_2\text{Cr}_2\text{O}_7$.

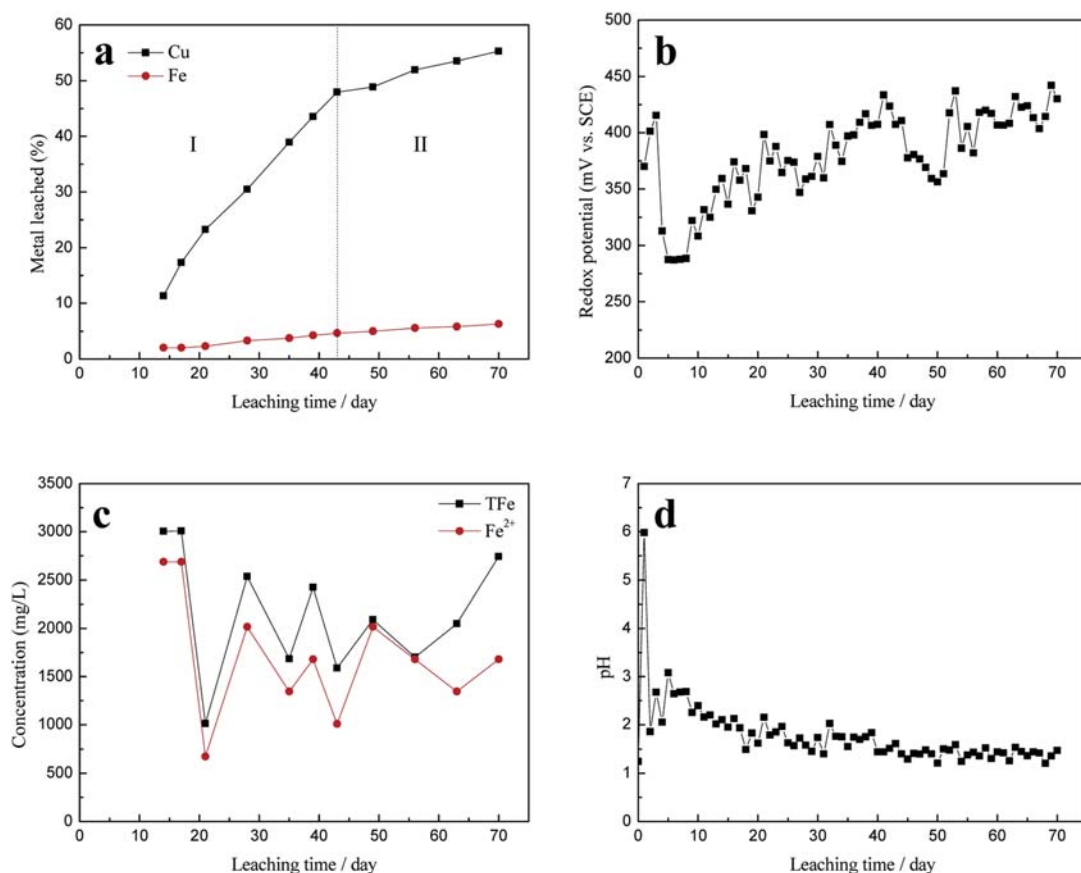


FIGURE 3.29 Metal leached (A), redox potentials (B), concentrations of total dissolved iron and Fe^{2+} (C), and pH (D) in leach liquors during the bioleaching of copper-bearing sulfide ore by moderately thermophilic microorganisms. From Qin, W., Yang, C., Lai, S., Wang, J., Liu, K., & Zhang, B. (2013). Bioleaching of chalcopyrite by moderately thermophilic microorganisms. *Bioresource Technology*, 129, 200–208. <https://doi.org/10.1016/j.biortech.2012.11.050>.

Cu^{2+} replaced Fe in a chalcopyrite lattice and was converted into covellite; thus, a decrease in copper concentration was observed. The Fe^{2+} and Fe^{3+} had a key role in forming chalcocite during the leaching of chalcopyrite with Cu^{2+} , and the Fe^{2+} ions significantly accelerated the leaching of chalcopyrite. The optimum mixed potential in the chemical leaching and bioleaching of chalcopyrite was accurately predicted with a newly proposed mathematic model, which was mainly determined by the concentrations of Cu^{2+} and Fe^{2+} . The model indicated that the mixed potential should be controlled at an optimum range ($E_L \sim E_H$) to obtain high leaching efficiency.

The electronic structure has an important role in the oxidation or reduction of chalcopyrite. Empty states of oxidizing agent overlap with filled states of chalcopyrite, or filled states of reducing agent overlap with empty states of chalcopyrite, which correspond to the strongly enabled oxidation and reduction of chalcopyrite, so higher copper extraction was obtained.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No. 51904339), National Basic Research Program of China (Grant No. 2010CB630905), High-Tech Research and Development Program of China (Grant No. 2012AA061501). I also thank Prof. Wenqing Qin and Jun Wang for their valuable advice.

References

- Acres, R. G., Harmer, S. L., & Beattie, D. A. (2010a). Synchrotron XPS, NEXAFS, and ToF-SIMS studies of solution exposed chalcopyrite and heterogeneous chalcopyrite with pyrite. *Minerals Engineering*, 23(11–13), 928–936. <https://doi.org/10.1016/j.mineng.2010.03.007>.
- Acres, R. G., Harmer, S. L., & Beattie, D. A. (2010b). Synchrotron XPS studies of solution exposed chalcopyrite, bornite, and heterogeneous chalcopyrite with bornite. *International Journal of Mineral Processing*, 94(1–2), 43–51. <https://doi.org/10.1016/j.minpro.2009.11.006>.
- Ahmadi, A., Schaffie, M., Manafi, Z., & Ranjbar, M. (2010). Electrochemical bioleaching of high grade chalcopyrite flotation concentrates in a stirred bioreactor. *Hydrometallurgy*, 104(1), 99–105. <https://doi.org/10.1016/j.hydromet.2010.05.001>.
- Arce, E. M., & González, I. (2002). A comparative study of electrochemical behavior of chalcopyrite, chalcocite and bornite in sulfuric acid solution. *International Journal of Mineral Processing*, 67(1–4), 17–28. [https://doi.org/10.1016/S0301-7516\(02\)00003-0](https://doi.org/10.1016/S0301-7516(02)00003-0).
- Bard, A. J., & Faulkner, L. R. (2001). *Electrochemical methods: Fundamentals and applications*.
- Brion, D. (1980). Etude par spectroscopie de photoelectrons de la degradation superficielle de FeS₂, CuFeS₂, ZnS et PbS a l'air et dans l'eau. *Applications of Surface Science*, 5(2), 133–152. [https://doi.org/10.1016/0378-5963\(80\)90148-8](https://doi.org/10.1016/0378-5963(80)90148-8).
- Cao, C. (1990). On the impedance plane displays for irreversible electrode reactions based on the stability conditions of the steady-state-I. One state variable besides electrode potential. *Electrochimica Acta*, 35(5), 831–836. [https://doi.org/10.1016/0013-4686\(90\)90077-D](https://doi.org/10.1016/0013-4686(90)90077-D).
- Chawla, S. K., Sankarraman, N., & Payer, J. H. (1992). Diagnostic spectra for XPS analysis of CuOSH compounds. *Journal of Electron Spectroscopy and Related Phenomena*, 61(1), 1–18. [https://doi.org/10.1016/0368-2048\(92\)80047-C](https://doi.org/10.1016/0368-2048(92)80047-C).
- Chen, J. H., Lan, L. H., & Liao, X. J. (2013). Depression effect of pseudo glycolythiourea acid in flotation separation of copper-molybdenum. *Transactions of Nonferrous Metals Society of China*, 23(3), 824–831. [https://doi.org/10.1016/S1003-6326\(13\)62535-2](https://doi.org/10.1016/S1003-6326(13)62535-2).
- Chen, J., Long, X., & Chen, Y. (2014). Comparison of multilayer water adsorption on the hydrophobic galena (PbS) and hydrophilic pyrite (FeS₂) surfaces: A DFT study. *Journal of Physical Chemistry C*, 118(22), 11657–11665. <https://doi.org/10.1021/jp5000478>.
- Chen, L., Zhou, Y., Krause, S., Munoz, A. G., Kunze, J., & Schmuki, P. (2008). Repair of thin thermally grown silicon dioxide by anodic oxidation. *Electrochimica Acta*, 53(8), 3395–3402. <https://doi.org/10.1016/j.electacta.2007.11.065>.
- Chusuei, C. C., Brookshier, M. A., & Goodman, D. W. (1999). Correlation of relative X-ray photoelectron spectroscopy shake-up intensity with CuO particle size. *Langmuir*, 15(8), 2806–2808. <https://doi.org/10.1021/la9815446>.
- Córdoba, E. M., Muñoz, J. A., Blázquez, M. L., González, F., & Ballester, A. (2008a). Leaching of chalcopyrite with ferric ion. Part II: Effect of redox potential. *Hydrometallurgy*, 93(3–4), 88–96. <https://doi.org/10.1016/j.hydromet.2008.04.016>.
- Córdoba, E. M., Muñoz, J. A., Blázquez, M. L., González, F., & Ballester, A. (2008b). Leaching of chalcopyrite with ferric ion. Part IV: The role of redox potential in the presence of mesophilic and thermophilic bacteria. *Hydrometallurgy*, 93(3–4), 106–115. <https://doi.org/10.1016/j.hydromet.2007.11.005>.
- Crundwell, F. K. (1988). The influence of the electronic structure of solids on the anodic dissolution and leaching of semi-conducting sulphide minerals. *Hydrometallurgy*, 21(2), 155–190. [https://doi.org/10.1016/0304-386X\(88\)90003-5](https://doi.org/10.1016/0304-386X(88)90003-5).
- Crundwell, F. K. (2013). The dissolution and leaching of minerals: Mechanisms, myths and misunderstandings. *Hydrometallurgy*, 139, 132–148. <https://doi.org/10.1016/j.hydromet.2013.08.003>.
- Crundwell, F. K. (2015a). The semiconductor mechanism of dissolution and the pseudo-passivation of chalcopyrite. *Canadian Metallurgical Quarterly*, 54(3), 279–288. <https://doi.org/10.1179/1879139515Y.0000000007>.

- Crundwell, F. K., Van Aswegen, A., Bryson, L. J., Biley, C., Craig, D., Marsicano, V. D., et al. (2015b). The effect of visible light on the dissolution of natural chalcopyrite (CuFeS_2) in sulphuric acid solutions. *Hydrometallurgy*, 158, 119–131. <https://doi.org/10.1016/j.hydromet.2015.10.014>.
- De Lima, G. F., De Oliveira, C., De Abreu, H. A., & Duarte, H. A. (2011). Water adsorption on the reconstructed (001) chalcopyrite surfaces. *Journal of Physical Chemistry C*, 115(21), 10709–10717. <https://doi.org/10.1021/jp201106e>.
- De Oliveira, C., De Lima, G. F., De Abreu, H. A., & Duarte, H. A. (2012). Reconstruction of the chalcopyrite surfaces-A DFT study. *Journal of Physical Chemistry C*, 116(10), 6357–6366. <https://doi.org/10.1021/jp300713z>.
- D'Hugues, P., Foucher, S., Gallé-Cavalloni, P., & Morin, D. (2002). Continuous bioleaching of chalcopyrite using a novel extremely thermophilic mixed culture. *International Journal of Mineral Processing*, 66(1–4), 107–119. [https://doi.org/10.1016/S0301-7516\(02\)00004-2](https://doi.org/10.1016/S0301-7516(02)00004-2).
- Eghbalian, M., & Dixon, D. G. (2011). Electrochemical study of leached chalcopyrite using solid paraffin-based carbon paste electrodes. *Hydrometallurgy*, 110(1–4), 1–12. <https://doi.org/10.1016/j.hydromet.2011.07.009>.
- Elsherief, A. E. (2002). The influence of cathodic reduction, Fe^{2+} and Cu^{2+} ions on the electrochemical dissolution of chalcopyrite in acidic solution. *Minerals Engineering*, 15(4), 215–223. [https://doi.org/10.1016/S0892-6875\(01\)00208-4](https://doi.org/10.1016/S0892-6875(01)00208-4).
- Farquhar, M. L., Wincott, P. L., Wogelius, R. A., & Vaughan, D. J. (2003). Electrochemical oxidation of the chalcopyrite surface: An XPS and AFM study in solution at pH 4. *Applied Surface Science*, 218(1–4), 34–43. [https://doi.org/10.1016/S0169-4332\(03\)00538-5](https://doi.org/10.1016/S0169-4332(03)00538-5).
- Gericke, M., Govender, Y., & Pinches, A. (2010). Tank bioleaching of low-grade chalcopyrite concentrates using redox control. *Hydrometallurgy*, 104(3–4), 414–419. <https://doi.org/10.1016/j.hydromet.2010.02.024>.
- Gerson, A. R., & Bredow, T. (2000). Interpretation of sulphur 2p XPS spectra in sulfide minerals by means of ab initio calculations. *Surface and Interface Analysis*, 29(2), 145–150. [https://doi.org/10.1002/\(SICI\)1096-9918\(200002\)29:2<145::AID-SIA727>3.0.CO;2-N](https://doi.org/10.1002/(SICI)1096-9918(200002)29:2<145::AID-SIA727>3.0.CO;2-N).
- Ghahremaninezhad, A., Asselin, E., & Dixon, D. G. (2011). Electrodeposition and growth mechanism of copper sulfide nanowires. *Journal of Physical Chemistry C*, 115(19), 9320–9334. <https://doi.org/10.1021/jp108283z>.
- Ghahremaninezhad, A., Dixon, D. G., & Asselin, E. (2012). Kinetics of the ferric-ferrous couple on anodically passivated chalcopyrite (CuFeS_2) electrodes. *Hydrometallurgy*, 125–126, 42–49. <https://doi.org/10.1016/j.hydromet.2012.05.004>.
- Ghahremaninezhad, A., Dixon, D. G., & Asselin, E. (2013). Electrochemical and XPS analysis of chalcopyrite (CuFeS_2) dissolution in sulfuric acid solution. *Electrochimica Acta*, 87, 97–112. <https://doi.org/10.1016/j.electacta.2012.07.119>.
- Goh, S. W., Buckley, A. N., & Lamb, R. N. (2006). Copper(II) sulfide? *Minerals Engineering*, 19(2), 204–208. <https://doi.org/10.1016/j.mineng.2005.09.003>.
- Goh, S. W., Buckley, A. N., Lamb, R. N., Rosenberg, R. A., & Moran, D. (2006). The oxidation states of copper and iron in mineral sulfides, and the oxides formed on initial exposure of chalcopyrite and bornite to air. *Geochimica et Cosmochimica Acta*, 70(9), 2210–2228. <https://doi.org/10.1016/j.gca.2006.02.007>.
- Gómez, C., Figueroa, M., Muñoz, J., Blázquez, M. L., & Ballester, A. (1996). Electrochemistry of chalcopyrite. *Hydrometallurgy*, 43(1–3), 331–344. [https://doi.org/10.1016/0304-386x\(96\)00010-2](https://doi.org/10.1016/0304-386x(96)00010-2).
- Gu, G., Hu, K., Zhang, X., Xiong, X., & Yang, H. (2013). The stepwise dissolution of chalcopyrite bioleached by *Lep-tospirillum ferriphilum*. *Electrochimica Acta*, 103, 50–57. <https://doi.org/10.1016/j.electacta.2013.04.051>.
- Harmer, S. L., Pratt, A. R., Nesbitt, H. W., & Fleet, M. E. (2005). Reconstruction of fracture surfaces on bornite. *The Canadian Mineralogist*, 43(5), 1619–1630. <https://doi.org/10.2113/gscanmin.43.5.1619>.
- Harmer, S. L., Thomas, J. E., Fornasiero, D., & Gerson, A. R. (2006). The evolution of surface layers formed during chalcopyrite leaching. *Geochimica et Cosmochimica Acta*, 70(17), 4392–4402. <https://doi.org/10.1016/j.gca.2006.06.1555>.
- He, H., Xia, J. L., Yang, Y., Jiang, H., Xiao, C. q., Zheng, L., et al. (2009). Sulfur speciation on the surface of chalcopyrite leached by *Acidianus manzaensis*. *Hydrometallurgy*, 99(1–2), 45–50. <https://doi.org/10.1016/j.hydromet.2009.06.004>.
- Hiroyoshi, N., Hirota, M., Hirajima, T., & Tsunekawa, M. (1997). A case of ferrous sulfate addition enhancing chalcopyrite leaching. *Hydrometallurgy*, 47(1), 37–45. [https://doi.org/10.1016/S0304-386X\(97\)00032-7](https://doi.org/10.1016/S0304-386X(97)00032-7).
- Hiroyoshi, N., Kuroiwa, S., Miki, H., Tsunekawa, M., & Hirajima, T. (2004). Synergistic effect of cupric and ferrous ions on active-passive behavior in anodic dissolution of chalcopyrite in sulfuric acid solutions. *Hydrometallurgy*, 74(1–2), 103–116. <https://doi.org/10.1016/j.hydromet.2004.01.003>.
- Hiroyoshi, N., Miki, H., Hirajima, T., & Tsunekawa, M. (2000). Model for ferrous-promoted chalcopyrite leaching. *Hydrometallurgy*, 57(1), 31–38. [https://doi.org/10.1016/S0304-386X\(00\)00089-X](https://doi.org/10.1016/S0304-386X(00)00089-X).

- Hiroyoshi, N., Miki, H., Hirajima, T., & Tsunekawa, M. (2001). Enhancement of chalcopyrite leaching by ferrous ions in acidic ferric sulfate solutions. *Hydrometallurgy*, 60(3), 185–197. [https://doi.org/10.1016/S0304-386X\(00\)00155-9](https://doi.org/10.1016/S0304-386X(00)00155-9).
- Holmes, P. R., & Crundwell, F. K. (2013). Polysulfides do not cause passivation: Results from the dissolution of pyrite and implications for other sulfide minerals. *Hydrometallurgy*, 139, 101–110. <https://doi.org/10.1016/j.hydromet.2013.07.006>.
- Jiang, C., Zhang, W., Zou, G., Xu, L., Yu, W., & Qian, Y. (2005). Hydrothermal fabrication of copper sulfide nanocones and nanobelts. *Materials Letters*, 59(8–9), 1008–1011. <https://doi.org/10.1016/j.matlet.2004.11.046>.
- Jones, D. L. (1974). *The leaching of chalcopyrite*. Vancouver: University of British Columbia.
- Jones, D. L., & Peters, E. (1976). *Leaching of chalcopyrite with ferric sulfate and ferric chloride* (pp. 633–653). Metall Soc of AIME.
- Jorcin, J. B., Orazem, M. E., Pébère, N., & Tribollet, B. (2006). CPE analysis by local electrochemical impedance spectroscopy. *Electrochimica Acta*, 51(8–9), 1473–1479. <https://doi.org/10.1016/j.electacta.2005.02.128>.
- Kinnunen, P. H. M., Heimala, S., Riekkola-Vanhanen, M. L., & Puhakka, J. A. (2006). Chalcopyrite concentrate leaching with biologically produced ferric sulphate. *Bioresource Technology*, 97(14), 1727–1734. <https://doi.org/10.1016/j.biortech.2005.07.016>.
- Klauber, C. (2003). Fracture-induced reconstruction of a chalcopyrite (CuFeS_2) surface. *Surface and Interface Analysis*, 35(5), 415–428. <https://doi.org/10.1002/sia.1539>.
- Klauber, C. (2008). A critical review of the surface chemistry of acidic ferric sulphate dissolution of chalcopyrite with regards to hindered dissolution. *International Journal of Mineral Processing*, 86(1–4), 1–17. <https://doi.org/10.1016/j.minpro.2007.09.003>.
- Klauber, C., Parker, A., Van Bronswijk, W., & Watling, H. (2001). Sulphur speciation of leached chalcopyrite surfaces as determined by x-ray photoelectron spectroscopy. *International Journal of Mineral Processing*, 62(1–4), 65–94. [https://doi.org/10.1016/S0301-7516\(00\)00045-4](https://doi.org/10.1016/S0301-7516(00)00045-4).
- Laajalehto, K., Kartio, I., & Nowak, P. (1994). XPS study of clean metal sulfide surfaces. *Applied Surface Science*, 81(1), 11–15. [https://doi.org/10.1016/0169-4332\(94\)90080-9](https://doi.org/10.1016/0169-4332(94)90080-9).
- Lee, M. S., Nicol, M. J., & Basson, P. (2008). Cathodic processes in the leaching and electrochemistry of covellite in mixed sulfate-chloride media. *Journal of Applied Electrochemistry*, 38(3), 363–369. <https://doi.org/10.1007/s10800-007-9447-5>.
- Li, Y., Chandra, A. P., & Gerson, A. R. (2014). Scanning photoelectron microscopy studies of freshly fractured chalcopyrite exposed to O_2 and H_2O . *Geochimica et Cosmochimica Acta*, 133, 372–386. <https://doi.org/10.1016/j.gca.2014.02.037>.
- Li, Y., Kawashima, N., Li, J., Chandra, A. P., & Gerson, A. R. (2013). A review of the structure, and fundamental mechanisms and kinetics of the leaching of chalcopyrite. *Advances in Colloid and Interface Science*, 197–198, 1–32. <https://doi.org/10.1016/j.cis.2013.03.004>.
- Liang, C. L., Xia, J. L., Yang, Y., Nie, Z. Y., Zhao, X. J., Zheng, L., et al. (2011). Characterization of the thermo-reduction process of chalcopyrite at 65°C by cyclic voltammetry and XANES spectroscopy. *Hydrometallurgy*, 107(1–2), 13–21. <https://doi.org/10.1016/j.hydromet.2011.01.011>.
- Liu, H. C., Nie, Z. Y., Xia, J. L., Zhu, H. R., Yang, Y., Zhao, C. H., et al. (2015). Investigation of copper, iron and sulfur speciation during bioleaching of chalcopyrite by moderate thermophile *Sulfobacillus thermosulfidooxidans*. *International Journal of Mineral Processing*, 137, 1–8. <https://doi.org/10.1016/j.minpro.2015.02.008>.
- Liu, H. C., Xia, J. L., & Nie, Z. Y. (2015). Relatedness of Cu and Fe speciation to chalcopyrite bioleaching by *Acidithiobacillus ferrooxidans*. *Hydrometallurgy*, 156, 40–46. <https://doi.org/10.1016/j.hydromet.2015.05.013>.
- López-Juárez, A., Gutiérrez-Arenas, N., & Rivera-Santillán, R. E. (2006). Electrochemical behavior of massive chalcopyrite bioleached electrodes in presence of silver at 35°C. *Hydrometallurgy*, 83(1–4), 63–68. <https://doi.org/10.1016/j.hydromet.2006.03.039>.
- Majuste, D., Ciminelli, V. S. T., Osseo-Asare, K., Dantas, M. S. S., & Magalhães-Paniago, R. (2012). Electrochemical dissolution of chalcopyrite: Detection of bornite by synchrotron small angle X-ray diffraction and its correlation with the hindered dissolution process. *Hydrometallurgy*, 114–123. <https://doi.org/10.1016/j.hydromet.2011.11.003>.
- Mikhlin, Y. L., Tomashevich, Y. V., Asanov, I. P., Okotrub, A. V., Varnek, V. A., & Vyalikh, D. V. (2004). Spectroscopic and electrochemical characterization of the surface layers of chalcopyrite (CuFeS_2) reacted in acidic solutions. *Applied Surface Science*, 225(1–4), 395–409. <https://doi.org/10.1016/j.apsusc.2003.10.030>.
- Mishra, K. K., & Osseo-Asare, K. (1988). Aspects of the interfacial electrochemistry of semiconductor pyrite (FeS_2). *Journal of the Electrochemical Society*, 135(10), 2502–2509. <https://doi.org/10.1149/1.2095366>.

- Morrison, S. R. (1978). Study of semiconductors using electrochemical techniques. *Journal of Vacuum Science and Technology*, 15(4), 1417–1421. <https://doi.org/10.1116/1.569799>.
- Mott, N. F. (1947). The theory of the formation of protective oxide films on metals.—III. *Transactions of the Faraday Society*, 429–434. <https://doi.org/10.1039/TF9474300429>.
- Nakai, I., Sugitani, Y., Nagashima, K., & Niwa, Y. (1978). X-ray photoelectron spectroscopic study of copper minerals. *Journal of Inorganic and Nuclear Chemistry*, 40(5), 789–791. [https://doi.org/10.1016/0022-1902\(78\)80152-3](https://doi.org/10.1016/0022-1902(78)80152-3).
- Nava, D., & González, I. (2006). Electrochemical characterization of chemical species formed during the electrochemical treatment of chalcopyrite in sulfuric acid. *Electrochimica Acta*, 51(25), 5295–5303. <https://doi.org/10.1016/j.electacta.2006.02.005>.
- Nava, D., González, I., Leinen, D., & Ramos-Barrado, J. R. (2008). Surface characterization by X-ray photoelectron spectroscopy and cyclic voltammetry of products formed during the potentiostatic reduction of chalcopyrite. *Electrochimica Acta*, 53(14), 4889–4899. <https://doi.org/10.1016/j.electacta.2008.01.088>.
- Parker, A., Klauber, C., Kougianos, A., Watling, H. R., & Van Bronswijk, W. (2003). An X-ray photoelectron spectroscopy study of the mechanism of oxidative dissolution of chalcopyrite. *Hydrometallurgy*, 71(1–2), 265–276. [https://doi.org/10.1016/S0304-386X\(03\)00165-8](https://doi.org/10.1016/S0304-386X(03)00165-8).
- Peng, H., Yang, Y., Li, X., Qiu, G., Liu, X., Huang, J., et al. (2006). Structure analysis of 16S rDNA sequences from strains of *Acidithiobacillus ferrooxidans*. *Journal of Biochemistry and Molecular Biology*, 39(2), 178–182. <https://doi.org/10.5483/bmbrep.2006.39.2.178>.
- Petersen, J., & Dixon, D. G. (2006). Competitive bioleaching of pyrite and chalcopyrite. *Hydrometallurgy*, 83(1–4), 40–49. <https://doi.org/10.1016/j.hydromet.2006.03.036>.
- Qin, W., Yang, C., Lai, S., Wang, J., Liu, K., & Zhang, B. (2013). Bioleaching of chalcopyrite by moderately thermophilic microorganisms. *Bioresource Technology*, 129, 200–208. <https://doi.org/10.1016/j.biortech.2012.11.050>.
- Sandström, A., Shchukarev, A., & Paul, J. (2005). XPS characterisation of chalcopyrite chemically and bio-leached at high and low redox potential. *Minerals Engineering*, 18(5), 505–515. <https://doi.org/10.1016/j.mineng.2004.08.004>.
- Sasaki, K., Takatsugi, K., Ishikura, K., & Hirajima, T. (2010). Spectroscopic study on oxidative dissolution of chalcopyrite, enargite and tennantite at different pH values. *Hydrometallurgy*, 100(3–4), 144–151. <https://doi.org/10.1016/j.hydromet.2009.11.007>.
- Sasaki, K., Takatsugi, K., & Tuovinen, O. H. (2012). Spectroscopic analysis of the bioleaching of chalcopyrite by *Acidithiobacillus caldus*. *Hydrometallurgy*, 127–128, 116–120. <https://doi.org/10.1016/j.hydromet.2012.07.013>.
- Smart, R. S. C., Skinner, W. M., & Gerson, A. R. (1999). XPS of sulphide mineral surfaces: Metal-deficient, polysulphides, defects and elemental sulphur. *Surface and Interface Analysis*, 28(1), 101–105. [https://doi.org/10.1002/\(SICI\)1096-9918\(199908\)28:1<101::AID-SIA627>3.0.CO;2-O](https://doi.org/10.1002/(SICI)1096-9918(199908)28:1<101::AID-SIA627>3.0.CO;2-O).
- Stirling, A., Bernasconi, M., & Parrinello, M. (2003). Ab initio simulation of water interaction with the (100) surface of pyrite. *The Journal of Chemical Physics*, 118(19), 8917–8926. <https://doi.org/10.1063/1.1566936>.
- Third, K. A., Cord-Ruwisch, R., & Watling, H. R. (2000). Role of iron-oxidizing bacteria in stimulation or inhibition of chalcopyrite bioleaching. *Hydrometallurgy*, 57(3), 225–233. [https://doi.org/10.1016/S0304-386X\(00\)00115-8](https://doi.org/10.1016/S0304-386X(00)00115-8).
- Third, K. A., Cord-Ruwisch, R., & Watling, H. R. (2002). Control of the redox potential by oxygen limitation improves bacterial leaching of chalcopyrite. *Biotechnology and Bioengineering*, 78(4), 433–441. <https://doi.org/10.1002/bit.10184>.
- Velásquez, P., Leinen, D., Pascual, J., Ramos-Barrado, J. R., Grez, P., Gómez, H., et al. (2005). A chemical, morphological, and electrochemical (XPS, SEM/EDX, CV, and EIS) analysis of electrochemically modified electrode surfaces of natural chalcopyrite (CuFeS₂) and pyrite (FeS₂) in alkaline solutions. *Journal of Physical Chemistry B*, 109(11), 4977–4988. <https://doi.org/10.1021/jp048273u>.
- Vetter, K. J. (1971). General kinetics of passive layers on metals. *Electrochimica Acta*, 16(11), 1923–1937. [https://doi.org/10.1016/0013-4686\(71\)85147-2](https://doi.org/10.1016/0013-4686(71)85147-2).
- Vilcáez, J., & Inoue, C. (2009). Mathematical modeling of thermophilic bioleaching of chalcopyrite. *Minerals Engineering*, 22(11), 951–960. <https://doi.org/10.1016/j.mineng.2009.03.001>.
- Vilcáez, J., Suto, K., & Inoue, C. (2008). Bioleaching of chalcopyrite with thermophiles: temperature-pH-ORP dependence. *International Journal of Mineral Processing*, 88(1–2), 37–44. <https://doi.org/10.1016/j.minpro.2008.06.002>.
- Vilcáez, J., Yamada, R., & Inoue, C. (2009). Effect of pH reduction and ferric ion addition on the leaching of chalcopyrite at thermophilic temperatures. *Hydrometallurgy*, 96(1–2), 62–71. <https://doi.org/10.1016/j.hydromet.2008.08.003>.

- Von Oertzen, G. U., Harmer, S. L., & Skinner, W. M. (2006). XPS and ab initio calculation of surface states of sulfide minerals: Pyrite, chalcopyrite and molybdenite. *Molecular Simulation*, 32(Issue 15), 1207–1212. <https://doi.org/10.1080/08927020601081616>.
- Watling, H. R. (2013). Chalcopyrite hydrometallurgy at atmospheric pressure: 1. Review of acidic sulfate, sulfate-chloride and sulfate-nitrate process options. *Hydrometallurgy*, 140, 163–180. <https://doi.org/10.1016/j.hydromet.2013.09.013>.
- Wu, S. F., Yang, C. R., Qin, W. Q., Jiao, F., Wang, J., & Zhang, Y. S. (2015). Sulfur composition on surface of chalcopyrite during its bioleaching at 50°C. *Transactions of Nonferrous Metals Society of China*, 25(12), 4110–4118. [https://doi.org/10.1016/S1003-6326\(15\)64062-6](https://doi.org/10.1016/S1003-6326(15)64062-6).
- Xia, J.L., Yang, Y., He, H., Liang, C.L., Zhao, X.J., Zheng, L., et al. (2010). Investigation of the sulfur speciation during chalcopyrite leaching by moderate thermophile *Sulfobacillus thermosulfidooxidans*. *International Journal of Mineral Processing*, 94(1–2), 52–57. <https://doi.org/10.1016/j.minpro.2009.11.005>.
- Yang, Y., Harmer, S., & Chen, M. (2014). Synchrotron X-ray photoelectron spectroscopic study of the chalcopyrite leached by moderate thermophiles and mesophiles. *Minerals Engineering*, 69, 185–195. <https://doi.org/10.1016/j.mineng.2014.08.011>.
- Yang, Y., Harmer, S., & Chen, M. (2015). Synchrotron-based XPS and NEXAFS study of surface chemical species during electrochemical oxidation of chalcopyrite. *Hydrometallurgy*, 156, 89–98. <https://doi.org/10.1016/j.hydromet.2015.05.011>.
- Yang, Y., Liu, W., Bhargava, S. K., Zeng, W., & Chen, M. (2016). A XANES and XRD study of chalcopyrite bioleaching with pyrite. *Minerals Engineering*, 157–162. <https://doi.org/10.1016/j.mineng.2016.01.019>.
- Yang, Y., Liu, W., & Chen, M. (2013). A copper and iron K-edge XANES study on chalcopyrite leached by mesophiles and moderate thermophiles. *Minerals Engineering*, 31–35. <https://doi.org/10.1016/j.mineng.2013.01.010>.
- Yang, Y., Liu, W., & Chen, M. (2015). XANES and XRD study of the effect of ferrous and ferric ions on chalcopyrite bioleaching at 30°C and 48°C. *Minerals Engineering*, 70, 99–108. <https://doi.org/10.1016/j.mineng.2014.08.021>.
- Yang, C., Qin, W., Lai, S., Wang, J., Zhang, Y., Jiao, F., et al. (2011). Bioleaching of a low grade nickel-copper-cobalt sulfide ore. *Hydrometallurgy*, 106(1–2), 32–37. <https://doi.org/10.1016/j.hydromet.2010.11.013>.
- Yin, Q., Kelsall, G. H., Vaughan, D. J., & England, K. E. R. (1995). Atmospheric and electrochemical oxidation of the surface of chalcopyrite (CuFeS₂). *Geochimica et Cosmochimica Acta*, 59(6), 1091–1100. [https://doi.org/10.1016/0016-7037\(95\)00026-V](https://doi.org/10.1016/0016-7037(95)00026-V).
- Yin, M., Wu, C. K., Lou, Y., Burda, C., Koberstein, J. T., Zhu, Y., et al. (2005). Copper oxide nanocrystals. *Journal of the American Chemical Society*, 127(26), 9506–9511. <https://doi.org/10.1021/ja050006u>.
- Zeng, W., Qiu, G., Zhou, H., & Chen, M. (2011). Electrochemical behaviour of massive chalcopyrite electrodes bioleached by moderately thermophilic microorganisms at 48°C. *Hydrometallurgy*, 105(3–4), 259–263. <https://doi.org/10.1016/j.hydromet.2010.10.012>.
- Zhao, H. B., Hu, M. H., Li, Y. N., Zhu, S., Qin, W. Q., Qiu, G. Z., et al. (2015). Comparison of electrochemical dissolution of chalcopyrite and bornite in acid culture medium. *Transactions of Nonferrous Metals Society of China*, 25(1), 303–313. [https://doi.org/10.1016/S1003-6326\(15\)63605-6](https://doi.org/10.1016/S1003-6326(15)63605-6).
- Zhao, H., Wang, J., Qin, W., Hu, M., Zhu, S., & Qiu, G. (2015). Electrochemical dissolution process of chalcopyrite in the presence of mesophilic microorganisms. *Minerals Engineering*, 71, 159–169. <https://doi.org/10.1016/j.mineng.2014.10.025>.
- Zhao, H. B., Wang, J., Qin, W. Q., Zheng, X. H., Tao, L., Gan, X. W., et al. (2015). Surface species of chalcopyrite during bioleaching by moderately thermophilic bacteria. *Transactions of Nonferrous Metals Society of China*, 25(8), 2725–2733. [https://doi.org/10.1016/S1003-6326\(15\)63897-3](https://doi.org/10.1016/S1003-6326(15)63897-3).
- Zhao, H., Wang, J., Yang, C., Hu, M., Gan, X., Tao, L., et al. (2015). Effect of redox potential on bioleaching of chalcopyrite by moderately thermophilic bacteria: An emphasis on solution compositions. *Hydrometallurgy*, 151, 141–150. <https://doi.org/10.1016/j.hydromet.2014.11.009>.
- Zhu, W., Xia, J.L., Yang, Y., Nie, Z.y., Zheng, L., Ma, C.y., et al. (2011). Sulfur oxidation activities of pure and mixed thermophiles and sulfur speciation in bioleaching of chalcopyrite. *Bioresource Technology*, 102(4), 3877–3882. <https://doi.org/10.1016/j.biortech.2010.11.090>.
- Zies, E. G., Allen, E. T., & Merwin, H. E. (1916). Some reactions involved in secondary copper sulphide enrichment. *Economic Geology*, 11(5), 407–503. <https://doi.org/10.2113/gsecongeo.11.5.407>.

Dissolution and passivation mechanism of chalcopyrite in bioleaching

4.1 Passivation mechanism of chalcopyrite bioleaching

4.1.1 Brief overview of passivating species

Large-scale commercial applications of chalcopyrite bioleaching, especially heap bioleaching of chalcopyrite, still develop slowly owing to the refractory nature of chalcopyrite. To overcome this challenge, first the dissolution process and passivation mechanism of chalcopyrite bioleaching should be studied to find the rate-limiting steps; then, the controlling method can be put forward. In addition, the oxidative dissolution of chalcopyrite by acidophilic microorganisms can contribute to the production of acid mine drainage (AMD), which severely contaminates water resources and soil. Hence, an understanding of the oxidative dissolution mechanism of chalcopyrite in the presence of acidophilic microorganisms would also be useful for prevention and remediation strategies for AMD sites.

The microorganisms used in chalcopyrite bioleaching have been generally classified into three categories according to their range of optimum growth temperature (mesophilic, moderately thermophilic, and extremely thermophilic), as shown in Table 4.1 (Johnson & Hallberg, 2003; Olson et al., 2003; Schippers, 2007; Rohwerder et al., 2003, pp. 239–248).

In the bioleaching system of mesophilic microorganisms, chalcopyrite is easily passivated, resulting in low dissolution kinetics and copper extraction. However, extremely thermophilic microorganisms (mainly including the extremely thermophilic Archaea) can significantly accelerate the dissolution kinetics of chalcopyrite. A large amount of acid and quantity of heat are generated in chalcopyrite bioleaching, and the extremely thermophilic Archaea has inherent tolerance to higher acidity, temperature, and metal ion concentrations. Although because of the lack of peptidoglycan the thermophilic Archaea do not have the rigid cell wall associated with bacteria and cannot withstand high shear force from the pulps, they are extraordinary for the future development of bioleaching chalcopyrite in heaps (Liu et al., 2016; Long et al., 2015). For these reasons, moderately thermophilic microorganisms have

TABLE 4.1 Typical microorganisms of chalcopyrite bioleaching.

Categories	Temperature optimum (°C)	Typical species
Mesophilic bacteria	<40	<i>Acidithiobacillus ferrooxidans</i> , <i>Acidithiobacillus thiooxidans</i> , and <i>Leptospirillum ferrooxidans</i>
Moderately thermophilic bacteria	40–55	<i>Acidimicrobium ferrooxidans</i> , <i>Sulfobacillus thermosulfidooxidans</i> , <i>Sulfobacillus acidophilus</i> , and <i>Acidithiobacillus caldus</i>
Extremely thermophilic Archaea	55–80	<i>Acidianus brierleyi</i> , <i>Metallosphaera sedula</i> , <i>Sulfolobus metallicus</i> , and <i>Sulfurococcus mirabilis</i>

wide application prospects (Olson et al., 2003; Pradhan et al., 2008; Rohwerder et al., 2003, pp. 239–248; Watling, 2006). Hence, the passivation and dissolution mechanisms of chalcopyrite bioleaching by moderately thermophilic microorganisms were mainly studied and summarized in this work.

The reported possible passivating species mainly consist of polysulfide (S_n^{2-}), elemental sulfur (S^0), and insoluble sulfate (SO_4^{2-}). There continues to be debates on this topic; here, we summarize and classify the viewpoints into different groups.

4.1.1.1 Polysulfide

Polysulfide, mainly metal-deficient polysulfide, may cause the significant passivation of chalcopyrite dissolution. Linge (1976) studied the electrochemical dissolution mechanism of chalcopyrite using a potentiometric titration technique. The researchers found that Fe dissolved before Cu at the beginning of dissolution, producing metal-deficient polysulfide. The poorly reactive and relatively dense Fe-deficient polysulfide with a low diffusion rate resulted in the passivation of chalcopyrite, and the thickness of the passive film of polysulfide was reported to be less than 1 μm (Parker et al., 1981; Hackl et al., 1995; Linge, 1976; Mustapha et al., 1977, pp. 161–177). Fu et al. (2012) claimed that polysulfide ($\text{Cu}_4\text{Fe}_2\text{S}_9$) was the main product causing the passivation of chalcopyrite in bioleaching by *Acidithiobacillus ferrooxidans*. Yang et al. (2013) analyzed the dissolution of chalcopyrite in the presence of mesophilic and moderately thermophilic microorganisms by X-ray absorption near edge structure (XANES). They proposed that Fe was more easily dissolved than Cu at the beginning of dissolution, producing polysulfide (CuS_n). At the later stage of bioleaching, CuS_n was converted to another type of polysulfide because of the further dissolution of Cu, resulting in the passivation of chalcopyrite. At 30°C, there was no significant amount of jarosite, but a large amount of jarosite was produced at 48°C. Chalcopyrite can be passivated in the potential range of 748–828 mV versus Standard hydrogen electrode (SHE), and there are two passivation regions nearby (Yang et al., 2015). Another work suggested that the dissolution kinetics of chalcopyrite was fast when the solution potential was less than 648 mV versus SHE. Chalcopyrite could not be leached effectively when the solution potential was higher than 698 mV versus SHE, and a large amount of elemental sulfur and jarosite was produced, but they were not the main causes of the passivation of chalcopyrite. At 673–898 mV versus SHE, the preferential dissolution of Fe led to the formation of Fe-deficient polysulfide on the

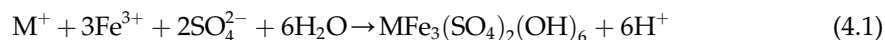
chalcopyrite surface and hindered ion diffusion from the interface of the chalcopyrite-passive film to the interface of the passive film/solution, resulting in passivation. The polysulfide was oxidized to elemental sulfur when the potential was higher than 898 mV versus SHE, but the dissolution of chalcopyrite could not be hindered until elemental sulfur accumulated to a certain amount on the chalcopyrite surface. The formed polysulfide was difficult to be directly oxidized by Fe^{3+} . Only the use of stronger oxidants (such as potassium dichromate and nitric acid) could quickly dissolve it (Congren et al., 2018). However, Klauber (2008) and Parker et al. (2003) proposed that polysulfide should not be the main passivating species because it is not stable.

4.1.1.2 Elemental sulfur

Dutrizac (1989) believed that elemental sulfur was the main passivating species of chalcopyrite in an ferric sulfate system. The formed elemental sulfur insulating layer may hinder the transfer of electrons at the chalcopyrite–liquid interface, which is the rate-limiting step determining the reduction of Fe^{3+} , inhibiting the redox reactions (Munoz et al., 1979). The elemental sulfur covers the surface of fresh chalcopyrite with several forms of monolayer elemental sulfur and multilayer elemental sulfur (Klauber et al., 2001). Sulfur arising from the S_2 dimers of chalcopyrite matrix may initially be oxidized to polymeric sulfur ($\mu\text{-S}$). The formed $\mu\text{-S}$ cannot be exploited by bacteria, but it can be converted to the orthorhombic allotrope $\alpha\text{-S}_8$, which is then oxidized to soluble sulfate by bacteria. Therefore, the rate-limiting step seems to be determined by the conversion rate of sulfur allotropes (Blight et al., 2009). Bevilaqua et al. (2004) found that the impedance of chalcopyrite changed owing to the adsorption of microorganisms on its surface in the first 30–60 min of bioleaching. After leaching for 71 h, the main components of the adsorption film were mainly composed of cells, biomolecules, and elemental sulfur, and the adsorption film acted as a capacitor, hindering the diffusion of ions and molecules on the chalcopyrite surface. In addition, elemental sulfur was the main product causing the passivation of chalcopyrite during bioleaching. Khoshkhoo et al. (2014) suggested that the content of elemental sulfur increased with the leaching time, and the formed elemental sulfur was not readily sublimated in the vacuum environment. The simultaneously produced nonstoichiometry FeOOH together with elemental sulfur formed a passive film that hindered the leaching of chalcopyrite. However, different studies proposed that elemental sulfur was not the passivation substance of chalcopyrite, and it was easily oxidized to sulfate (Liu et al., 2015; Sandström et al., 2005; Zhu et al., 2011).

4.1.1.3 Insoluble sulfate

Insoluble sulfate species mainly consisting of jarosite have also been widely reported as the main passivating species in chalcopyrite bioleaching (Eq. 4.1):



where M is a monovalent cation (e.g., H_3O^+ , Na^+ , K^+ and NH_4^+).

Sandström et al. (2005) examined the surface products (*Sulfolobus metallicus* at 65°C) using X-ray photoelectron spectroscopy (XPS). They found that elemental sulfur was oxidized to sulfate at a low potential and the passivation of chalcopyrite was mainly caused by jarosite. Similar results were obtained by some others (He, 2009; Zhu et al., 2011). Klauber (2008)

believed that the reactivity of polysulfide was strong, and it could be oxidized to elemental sulfur by oxygen. Elemental sulfur could cause the passivation of chalcopyrite, but it was easily peeled from the chalcopyrite surface, and jarosite should be the main passivating species. Electrons can be transferred to Fe^{3+} owing to disulfide oxidation through surface protonation and hydration reactions, producing thiosulfate. Thiosulfate can be further oxidized to ferric sulfates, which act as a crystal nucleus inducing the formation of jarosite, and eventually cause the passivation of chalcopyrite (Parker et al., 2003). Zeng (2011) suggested that the early stage of chalcopyrite bioleaching was an acid consumption process that required the continuous addition of acid to ensure a stable and low pH, and that the passivation layer was mainly composed of jarosite in chalcopyrite bioleaching by moderately thermophilic microorganisms. The relations among microorganism growth, the evolution of community structure, and passive film were studied by the Amplified Ribosomal DNA Restriction Analysis (ARDRA) method and polymerase chain reaction technique. The researchers found that the passive film notably hindered the growth of adsorbed microorganisms. A large amount of intermediate Cu–S was accumulated on the surface of chalcopyrite with the electrochemical reactions, but it was not stable because it was easily oxidized by Fe^{3+} . Thus, Cu–S did not cause the passivation of chalcopyrite; jarosite should be the main components of the passive film. Similar passivating species have also been reported in some publications (Córdoba et al., 2008; Sasaki et al., 2009; Zhang et al., 2014; Zhou et al., 2009). However, some studies proposed that chalcopyrite dissolution might not be hindered even though large amounts of jarosite were produced, and the loose jarosite was easily peeled from the surface of the mineral (Fu et al., 2012; Liu et al., 2015; Yang et al., 2015). In addition, $\text{Fe}_2\text{O}_3/\text{Fe}(\text{OH})_3/\text{FOOH}$ has been regarded as the passivating species of chalcopyrite bioleaching in some publications (Ahmadi et al., 2010; Khoshkhoo et al., 2014; Li & Huang, 2011). These species can act as an activating agent in the formation of jarosite (Casas, Lienqueo, Cubillos, & Herrera, 2000).

The main products causing chalcopyrite passivation are S-containing species. Therefore, it is important to analyze S-containing intermediates in chalcopyrite bioleaching.

4.1.2 Bioleaching behavior

A chalcopyrite sample was obtained from Daye, Hubei Province, China. X-ray diffraction analysis (XRD) showed that it was of high purity. The chemical analysis showed that the chalcopyrite sample contained 33.91% Cu, 30.62% Fe, 32.90% S, 0.039% Pb, and 0.018% Zn. Samples were splintered into small fragments with a geological hammer and dry-ground with porcelain ball milling. The product was screened to -0.074 mm for bioleaching experiments. All chemicals used were of analytical purity in this work. The mixed moderately thermophilic bacteria used in this study were obtained from a leaching solution sample from Inner Mongolia Province, China. Enrichment culture was performed in 250-mL shake flasks in an orbital incubator with a stirring speed of 160 rpm at 50°C . The basic culture medium was composed of the following compositions (Ni et al., 2014): 3.0 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.1 g/L KCl, 0.5 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g/L K_2HPO_4 , 0.01 g/L $\text{Ca}(\text{NO}_3)_2$, and 0.02% yeast extract. Three species (*Sulfobacillus thermosulfidooxidans*, *Acidithiobacillus caldus*, and *Ferroplasma* sp.) were detected by community structure analysis, as described by Qin et al. (2009). For leaching experiments, cells were inoculated into 250-mL flasks containing 100 mL of sterilized culture medium and 2 g of chalcopyrite. The initial cells concentration was 1.0×10^7 cells/mL. The flasks were placed in an orbital shaker at 160 rpm and 50°C . The parallel experiments without

cells but with the same culture medium and chalcopyrite were run as sterile experiments. All leaching experiments were supplemented with 0.02% yeast extract. The solution pH was controlled at 1.6–2.0 with diluted sulfuric acid. Water lost by evaporation was supplemented periodically by adding sterile water until the mass of the flask equaled its initial mass. Soluble Cu and Fe were determined by atomic absorption spectroscopy. The pH of the supernatant was measured with a pH meter (BPP-922, BELL) and the oxidation-reduction potential (ORP) in the leaching solution was measured with a Pt electrode with reference to a saturated Ag/AgCl electrode.

Fig. 4.1A shows that the percent extraction of Cu for the leaching by sterile control and microorganisms was 4.0% and 21.5%, respectively. In comparison, the Fe percent extraction was lower than Cu; only 3.8% and 10.5% Fe was leached by sterile control and microorganisms (Fig. 4.1B), respectively. The Fe percent extraction decreased owing to precipitation of soluble ferric iron as jarosite during bioleaching (Eq. 4.1) (Qin et al., 2013; Yang et al., 2011). Fig. 4.1C shows that the acid was consumed almost in the whole leaching process (the first 25 days), but it was produced as the formation of jarosite. The ORP of leaching with the microorganisms was reached and stabilized at about 630 mV owing to ferrous

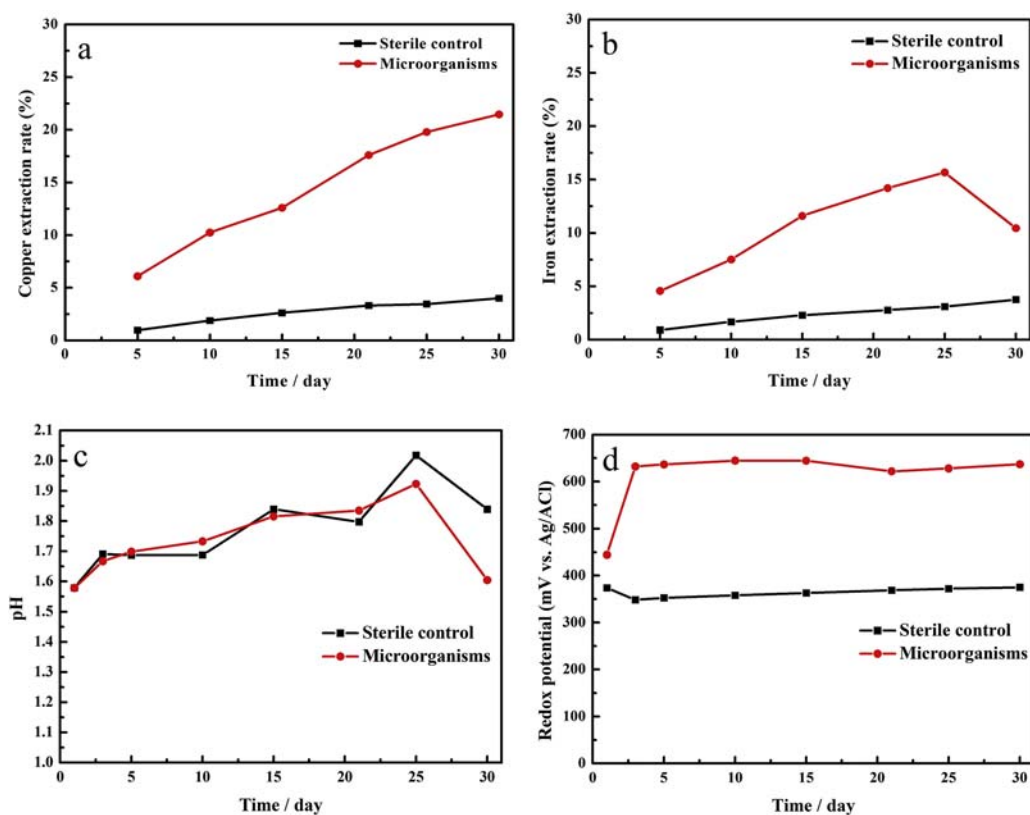


FIGURE 4.1 Copper extraction (A), iron extraction (B), pH (C), and oxidation-reduction potential (D) in leach liquors during the (bio)leaching of chalcopyrite. From Wu, S., Yang, C., Qin, W., Jiao, F., Wang, J., & Zhang, Y. (2015). Sulfur composition on surface of chalcopyrite during its bioleaching at 50 °C. *Transactions of Nonferrous Metals Society of China*, 25(12), 4110e4118. [https://doi.org/10.1016/S1003-6326\(15\)64062-6](https://doi.org/10.1016/S1003-6326(15)64062-6).

ions being oxidized to ferric ions by the microorganisms, whereas the ORP of leaching by sterile control stabilized at about 370 mV (Fig. 4.1D).

In a previous study (Qin et al., 2013), the author showed that the percent extraction of Cu for the bioleaching of chalcopyrite by a mixed moderately thermophilic culture under similar conditions was 92.5%, and concluded that the chalcopyrite dissolution rate depended on the ORP of the solution. Similarly, Hiroyoshi et al. (2001) found that the leaching rate was significantly enhanced by the addition of 0.50 mol/L Fe^{2+} and 0.01 mol/L Cu^{2+} together with 0.03 mol/L Fe^{3+} . Sandström et al. (2005) suggested that a low Electric potential of half cells (Eh) (around 620 mV vs. SHE) in sulfuric acid media was beneficial to chalcopyrite leaching compared with a higher Eh, such as 800 mV (SHE). To understand this phenomenon, Hiroyoshi et al. (2001) and Gu et al. (2013) proposed a two-step reaction model for chalcopyrite leaching at low ORP. First, chalcopyrite was reduced by ferrous to form chalcocite, and then the formed chalcocite was oxidized by ferric. Electrochemical studies showed that chalcocite might be a possible intermediate compound (Arce & González, 2002; Gu et al., 2013; Velásquez et al., 2005). Thus, dissolution of chalcopyrite would be favored when ORP was less than around 650 mV (SHE), or at an appropriate ratio of $[\text{Fe}^{3+}]/[\text{Fe}^{2+}]$ (Hiroyoshi et al., 2001; Qin et al., 2013; Sandström et al., 2005; Vilcáez et al., 2008, 2009).

4.1.3 Intermediate species analysis

The mineralogical compositions of solid samples and residues were examined by XRD (Rigaku Dmax-2000). Fig. 4.2 shows the XRD patterns of chalcopyrite and residues leached by sterile control and microorganisms. Peaks of tetragonal chalcopyrite were identified. Lesser amounts of jarosite formed during bioleaching (Fig. 4.2C).

XPS can provide reliable data to determine the trace amounts of intermediate species during bioleaching. In this work, XPS was conducted with an ESCALAB 250Xi spectrometer with monochromatic Al excitation (1486.6 eV) operating at 200 W. High-resolution core level spectra were collected using a pass energy of 20 eV and an energy step of 0.1 eV. The chamber pressure was set to the limit of 1×10^{-9} mbar. Binding energy calibration was based on C 1s at 284.6 eV. Fig. 4.3A–C shows the S 2p core level peak. The S 2p peaks also occur as doublets, S 2p_{3/2} and S 2p_{1/2}, as a result of spin–orbit splitting. The S 2p spectra were fitted using a 2:1 peak area ratio for S 2p_{3/2} to S 2p_{1/2} and 1.2-eV splitting. The peaks fitted to the spectra use a summed Gaussian–Lorentzian (SGL) function to describe the convoluted Gaussian–Lorentzian line shapes of the peaks. The weighting used was 70% Gaussian and 30% Lorentzian. The background of the spectrum was obtained using the Shirley method. Fig. 4.3A shows the S 2p core level peak of chalcopyrite, which consists of three major doublets, and the S 2p_{3/2} peaks of three doublets of Fig. 4.3A are centered at 161.3, 161.9, and 162.8 eV, respectively. It has been reported that the S 2p_{3/2} peak originating from chalcopyrite (i.e., monosulfide $[\text{S}^{2-}]$), centers at about 161.4 eV (Ghahremaninezhad et al., 2013; Nava et al., 2008). The low-intensity S 2p_{3/2} peaks centered at 161.9 and 162.8 eV are assigned to S_2^{2-} (Acres et al., 2010a, 2010b; Klauber, 2003) and S_n^{2-} (Acres et al., 2010a, 2010b), respectively. Many authors claimed that the S_2^{2-} species rapidly form a layer on freshly fractured chalcopyrite surfaces (Acres et al., 2010a, 2010b; Klauber, 2003; Klauber et al., 2001; Parker et al., 2003). de Oliveira et al. applied the Density Functional Theory (DFT)/plane waves methodology to study the surface of chalcopyrite reconstruction. Their results also showed the formation of S_2^{2-} and S_4^{2-} on chalcopyrite surfaces. In addition, a low-intensity 2p peak

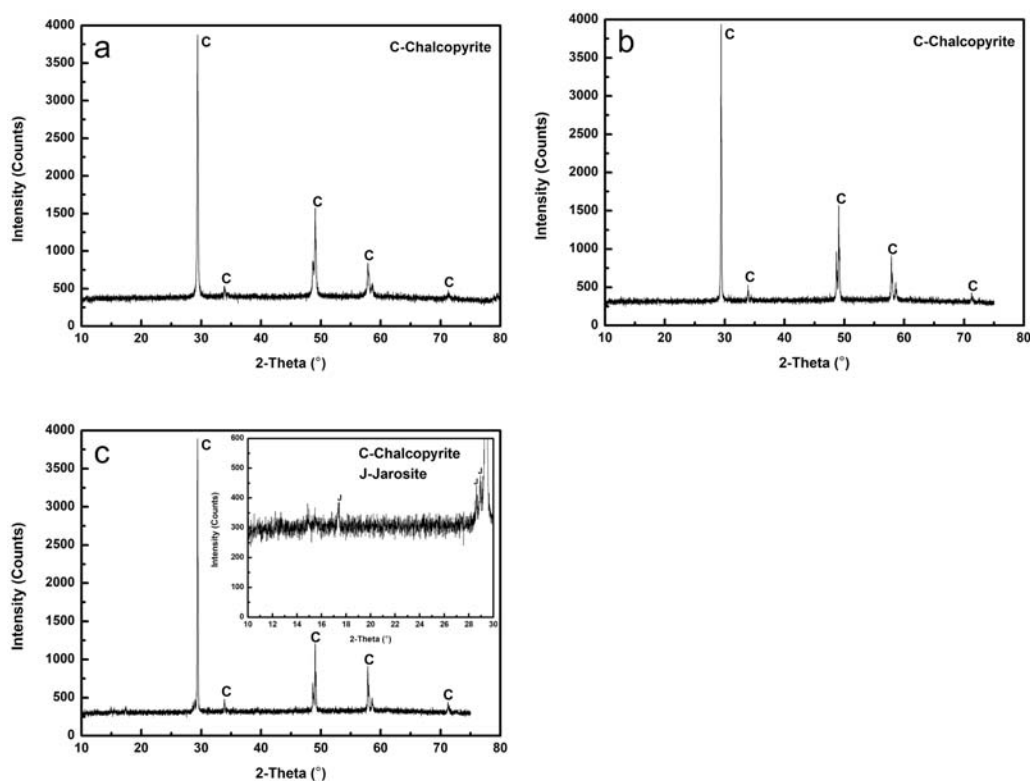


FIGURE 4.2 X-ray diffraction patterns of chalcopyrite (A), and residues leached by sterile control (B) and microorganisms (C). From Wu, S., Yang, C., Qin, W., Jiao, F., Wang, J., & Zhang, Y. (2015). Sulfur composition on surface of chalcopyrite during its bioleaching at 50 °C. *Transactions of Nonferrous Metals Society of China*, 25(12), 4110e4118. [https://doi.org/10.1016/S1003-6326\(15\)64062-6](https://doi.org/10.1016/S1003-6326(15)64062-6).

located at 164.3 eV was observed, which was considered to result from the energy loss owing to S 3p → Fe 3d excitation (Acres et al., 2010a, 2010b; Klauber, 2003).

Fig. 4.3B shows a high-resolution spectrum of S 2p for chalcopyrite leached by sterile control, with four fitting peaks centered at 161.3, 162.2, 163.3, and 164.1 eV, respectively, arising from the monosulfide (Ghahremaninezhad et al., 2013; Goh et al., 2006; Harmer et al., 2006; Li et al., 2010; Nava et al., 2008), polysulfide (Acres et al., 2010a, 2010b; Harmer et al., 2006), and sulfur (Gerson & Bredow, 2000; Harmer et al., 2006; Li et al., 2013; Smart et al., 1999). Fig. 4.3C shows a high-resolution spectrum of S 2p for chalcopyrite leached by microorganisms. Five fitting peaks centered at 161.2, 162.0, 163.1, 164.0, and 168.8 eV, respectively, arising from the monosulfide (Ghahremaninezhad et al., 2013; Goh et al., 2006; Harmer et al., 2006; Li et al., 2010; Nava et al., 2008), disulfide (Acres et al., 2010a, 2010b; Li et al., 2010), polysulfide (Acres et al., 2010a, 2010b; Harmer et al., 2006), sulfur (Gerson & Bredow, 2000; Harmer et al., 2006; Li et al., 2013; Smart et al., 1999), and sulfate (Klauber et al., 2001; Li et al., 2010; Li et al., 2013), and most of the sulfate were probably contributed by jarosite. The formation of S_2^{2-} and S_n^{2-} is due to the dissolution of Cu and Fe atoms from the crystal lattice of chalcopyrite. Fe atoms dissolve preferentially from the chalcopyrite lattice (Fig. 4.3D), and this result is in agreement with previous work (Ghahremaninezhad et al., 2013; Yin et al., 1995).

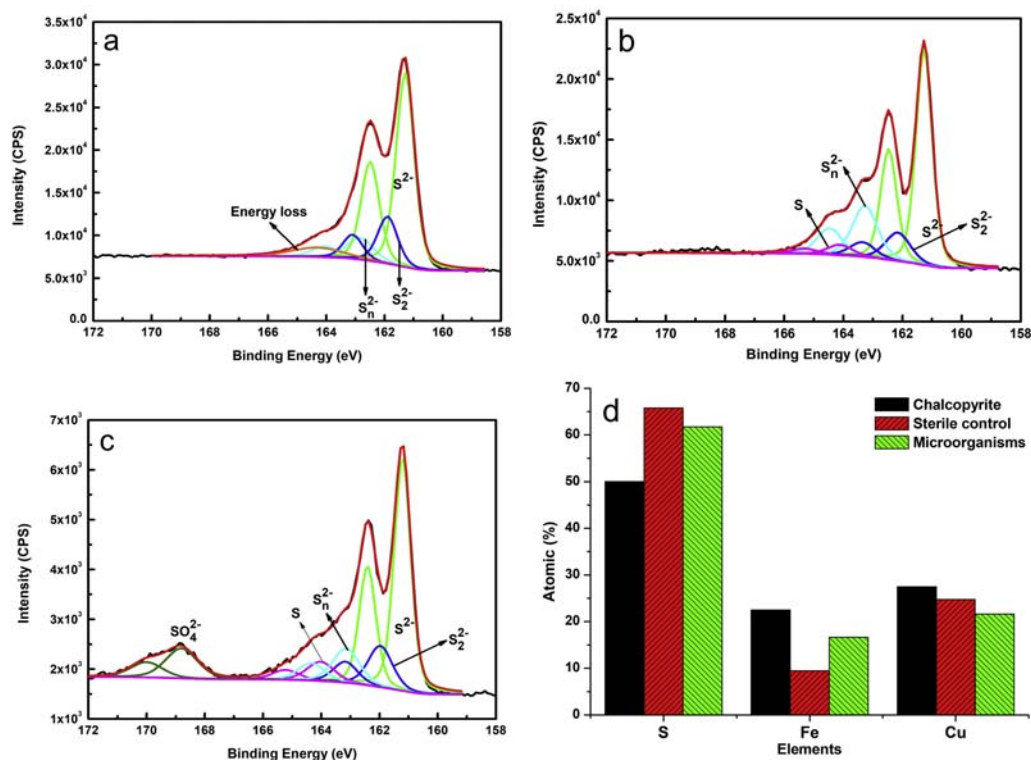


FIGURE 4.3 X-ray photoelectron spectroscopy spectrum of S 2p peaks of chalcopyrite exposed to air for no more than a few minutes (A), leached by sterile control (B), microorganisms (C), and composition of Cu, Fe, and S fraction on the surface of chalcopyrite before and after leaching (D). CPS, Counts per second. From Wu, S., Yang, C., Qin, W., Jiao, F., Wang, J., & Zhang, Y. (2015). Sulfur composition on surface of chalcopyrite during its bioleaching at 50 °C. *Transactions of Nonferrous Metals Society of China*, 25(12), 4110e4118. [https://doi.org/10.1016/S1003-6326\(15\)64062-6](https://doi.org/10.1016/S1003-6326(15)64062-6).

Fig. 4.4A shows a high-resolution spectrum of Cu 2p for original chalcopyrite and residue leaching by sterile control and microorganisms. The fitting results of peaks revealed that the Cu 2p_{3/2} peak of three samples was in the binding energy range of 932.0–932.2 eV, which was close to the binding energies for the Cu 2p_{3/2} in chalcopyrite (Brion, 1980; Goh et al., 2006; Nava et al., 2008). The presence of Cu²⁺ usually results in the appearance of a satellite peak around the binding energy of 942 eV (Chusuei et al., 1999; Ghahremaninezhad et al., 2013; Yin et al., 2005). Because the satellite peak is absent in the Cu 2p core level peaks of Fig. 4.4A, cupric is absent on the mineral surface even after it is leached by microorganisms.

The high-resolution spectrum of Fe 2p for chalcopyrite exposed to air for no more than a few minutes, leached by sterile control and microorganisms, is provided in Fig. 4.4B. The Fe 2p spectrum shows that the chalcopyrite was oxidized even exposure to air only for a few minutes. The peak near 708 eV can be assigned to Fe–S (Ghahremaninezhad et al., 2013). The peak in the binding energy range of 710–712 eV can be assigned to Fe(III) –O, –OH, or –OOH (Acres et al., 2010a, 2010b; Brion, 1980; Ghahremaninezhad et al., 2013; Harmer et al., 2006). For the bioleaching Fe 2p spectrum, the component near 712 eV binding energy can be assigned to jarosite.

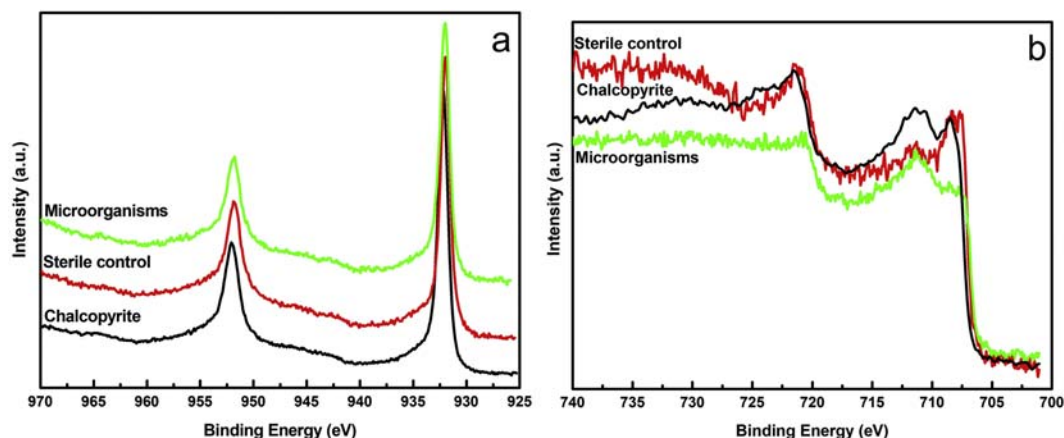


FIGURE 4.4 X-ray photoelectron spectroscopy spectrum of Cu (A) and Fe (B) 2p peak of chalcopyrite exposed to air for no more than a few minutes, leached by sterile control and microorganisms. From Wu, S., Yang, C., Qin, W., Jiao, F., Wang, J., & Zhang, Y. (2015). Sulfur composition on surface of chalcopyrite during its bioleaching at 50 °C. *Transactions of Nonferrous Metals Society of China*, 25(12), 4110e4118. [https://doi.org/10.1016/S1003-6326\(15\)64062-6](https://doi.org/10.1016/S1003-6326(15)64062-6).

4.1.4 Discussions on the passivation mechanism

During bioleaching, chalcopyrite dissolution is closely linked to the ORP. The lower ORP is conducive to the dissolution of chalcopyrite, whereas an inhibition layer (mostly referred to as a passivation layer) formed under high ORP can impede the further dissolution of chalcopyrite (Petersen & Dixon, 2006; Vilcáez et al., 2008; Zhao et al., 2015, 2019). It has been suggested that when the ORP is lower (between 350 and 480 mV [versus Ag/AgCl]), chalcopyrite is mainly converted to Cu_2S , accelerating the dissolution of chalcopyrite (Hiroyoshi et al., 2002). However, the detailed dissolution and inhibition mechanisms of chalcopyrite are still debated. To investigate the passivation mechanism of chalcopyrite, multiple electrochemical techniques were carried out, including the open circuit potential (OCP), cyclic voltammetry, Tafel and potentiostatic polarization, and electrochemical impedance spectroscopy (EIS). A chalcopyrite sample with high purity used as the work electrode was obtained from Guilin, Guangxi Province, China. Results showed that the OCP of the chalcopyrite electrode was around 217 mV (versus Ag/AgCl). Thus, a series of electrochemical techniques based on potentiostatic polarization with a potential of 250–700 mV (versus Ag/AgCl) with a gradient of 50 mV (versus Ag/AgCl) was performed.

Fig. 4.5 shows the $I-t$ curves of chalcopyrite electrode under different potentials. Results showed that under the conditions of 250–650 mV, the polarization currents showed significant differences only in the first 1.0 h; then they gradually tended to a slight stable value (about 10^{-7} to 10^{-6} A) with an increase in polarization time. This indicated that the chalcopyrite basically cannot dissolve owing to a low external potential or the formation of a passivation film under the condition of 250–650 mV. However, the polarization current of chalcopyrite stabilized at a large value (5.159×10^{-4} A) under 700 mV, which suggests that chalcopyrite was significantly dissolved under this condition.

Fig. 4.6 shows the equivalent circuit model for EIS fitting, and Fig. 4.7 shows the fitted R_1 (solution resistance), R_2 (electric double-layer resistance/charge transfer resistance), and R_3 (passivation layer resistance) of different groups. R_1 represents the solution resistance

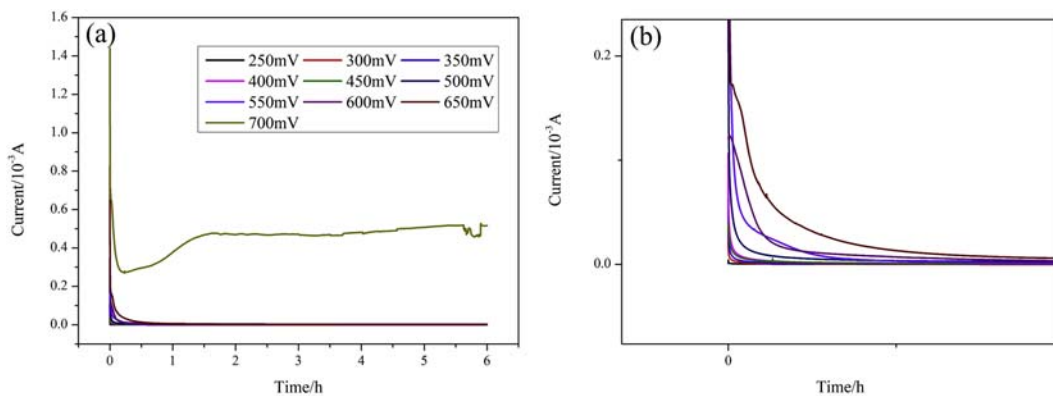


FIGURE 4.5 I-t curves of chalcopyrite electrode under different potentials.

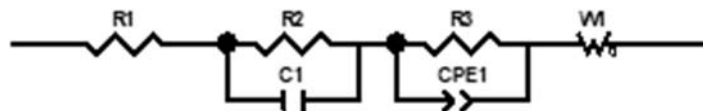


FIGURE 4.6 Equivalent circuit model for electrochemical impedance spectroscopy fitting. C, Capacitor; CPE, Constant phase element.

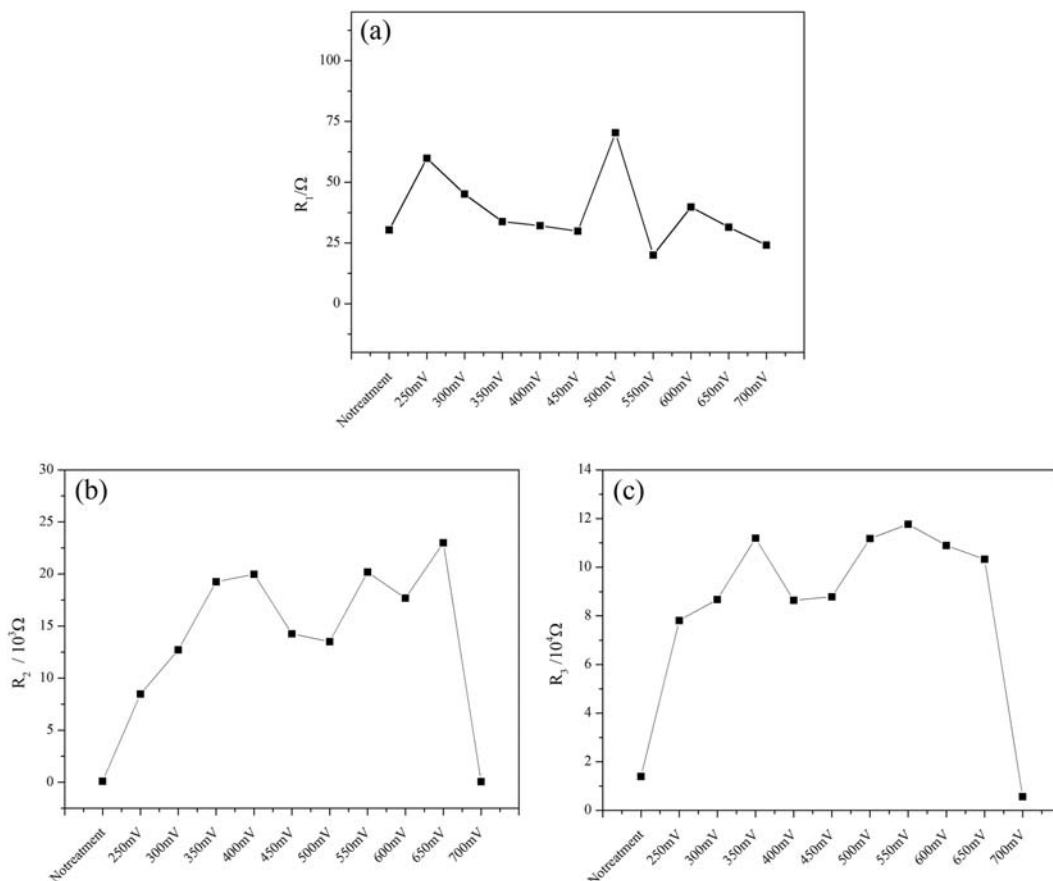


FIGURE 4.7 Fitted (A) R_1 (solution resistance), (B) R_2 (electric double-layer resistance/charge transfer resistance), and (C) R_3 (passivation layer resistance) of different groups.

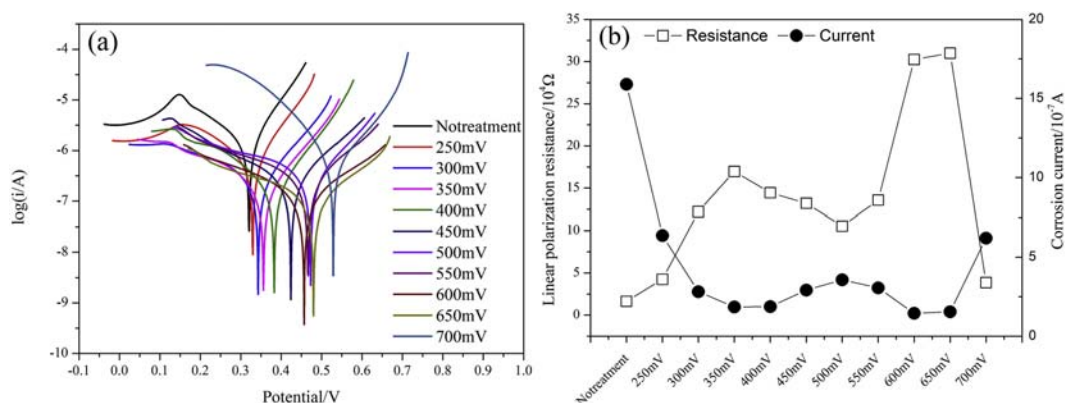


FIGURE 4.8 (A) Tafel plots; (B) Linear polarization resistance curve and corrosion current curve.

between the chalcopyrite electrode and the reference electrode, which was related to environmental parameters including the electrolyte, temperature, and the distance between the two electrodes. Fig. 4.7A shows that the R_1 of different groups all are about 50Ω , reflecting the high consistency of the experimental environment. Fig. 4.7B and C demonstrate the similar variations in trend of R_2 and R_3 . Untreated and 700-mV polarization groups obtained the similar smallest R_2 and R_3 , which indicated that chalcopyrite dissolved quickly at 700 and 700 mV might reach the transpassivation potential. The values of R_2 and R_3 under other potential conditions were all greater than the corresponding values of the untreated chalcopyrite, indicating that the chalcopyrite was passivated with different degrees after treatment at 250–650 mV. However, after treatment at 450 and 500 mV, the values of R_2 were relatively small, and after treatment at 400 and 450 mV, R_3 also exhibited smaller values, indicating that the R_2 and the R_3 of chalcopyrite reached relatively small values at about 450 mV. That also proved that an interval existed in the passivation range of 250–650 mV, in which chalcopyrite was relatively easily dissolved.

Fig. 4.8 shows the Tafel plots, fitted linear polarization resistance curve, and corrosion current curve. The polarization resistance was inversely proportional to the corrosion current. It had a variation trend similar to R_2 and R_3 , which suggests the transpassivation dissolution of chalcopyrite at 700 mV and the special interval between 450 and 550 mV where chalcopyrite was dissolved relatively quickly.

Combined with the results of the multiple electrochemical tests, the solubility of chalcopyrite under different potential conditions was proposed. When the potential was at 250–650 mV, the chalcopyrite was passivated, and 700 mV might reach the transpassivation potential. However, there is a special interval (around 450 mV) in the passivation range of 250–650 mV in which chalcopyrite was dissolved relatively quickly.

4.2 Effects of mineralogical properties on passivation and dissolution mechanism

4.2.1 Bioleaching behavior

Chalcopyrite samples from three different regions were designated chalcopyrite A, B, and C. They were obtained from the Chambishi Copper Mine of Zambia, Hunan Province of

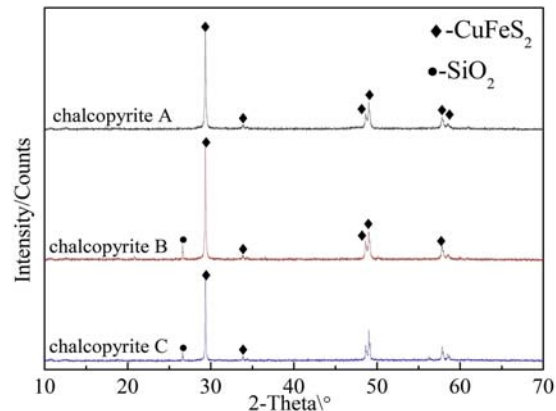


FIGURE 4.9 X-ray diffraction analysis of chosen chalcopyrite samples. From Zhao, H., Huang, X., Wang, J., Li, Y., Liao, R., Wang, X., Qiu, X., Xiong, Y., Qin, W., & Qiu, G. (2017). Comparison of bioleaching and dissolution process of *p*-type and *n*-type chalcopyrite. *Minerals Engineering*, 109, 153–161. <https://doi.org/10.1016/j.mineng.2017.03.013>.

China and Guangxi Province of China, respectively. XRD (DX-2700) (Fig. 4.9) shows that the three chalcopyrite samples were of extremely high purity (higher than 95%) without obvious diffraction peaks of impurities. An elemental analysis of chalcopyrite samples is presented in Table 4.2. Chalcopyrite A mainly contained 35.8% Cu, 27.3% Fe, and 29.4% S; chalcopyrite B mainly contained 31.9% Cu, 26.9% Fe, and 28.6% S; and chalcopyrite C mainly contained 30.1% Cu, 26.7% Fe, and 27.6% S. The Hall effects test (HMS-3000/0.55T) was used to analyze the semiconductor properties of chalcopyrite samples. The analytical results are presented in Table 4.3. The Hall effect was discovered in 1879 by Edwin Hall. It is caused by the nature of the current in a material (Hall, 1880). The material can experience the Lorentz force in the presence of a magnetic field owing to the movement of many small charge carriers in

TABLE 4.2 Chemical element analysis of chalcopyrite A, B, and C/%.

Chalcopyrite	Cu	Fe	S	Zn	Mg	Al	Mn	Bi	Si	O	Cl	K	Ca	Se
A	35.8	27.3	29.4	1.35	0.14	0.17	0.03	0.02	0.27	0.01	0.01	0.07	0.05	0.06
B	31.9	26.9	28.6	—	—	0.04	—	—	0.01	—	0.03	—	0.02	—
C	30.1	26.7	27.6	—	—	0.13	—	—	1.49	13.4	0.02	0.01	0.08	—

Cu, Fe, and S elements were analyzed by chemical analysis; the other elements were detected by X-ray fluorescence spectrometry.

TABLE 4.3 Results of Hall effect analysis of chalcopyrite samples.

Chalcopyrite	Nb (cm ⁻³)	μ (cm ² /V/s)	rho (Ω·cm)
A	7.01E+19	5.19E-02	1.715
B	−1.15E+18	1.362	3.999
C	−4.27E+18	1.75E-01	8.383

materials, typically electrons, holes, ions, or all three. When a semiconductor is located in a magnetic field, the charge carriers of the semiconductor can experience a force in a direction perpendicular to both the magnetic field and the current, and a voltage appears at the semiconductor edges (David, 2002; Ohgaki et al., 2008). Hence, the direction (positive or negative) of charge carrier concentration can be used to judge the types of semiconductor, and the carrier concentration can be used to evaluate the conductivity of the semiconductor. An N-type semiconductor mainly uses electrons as charge carriers and an p-type semiconductor principally uses holes as charge carriers. Therefore, chalcopyrite A belongs to p-type chalcopyrite, and high carrier concentrations ($7.01\text{E}+19\text{ cm}^{-3}$) reveal that this p-type chalcopyrite is sufficiently close to being degenerate and should act as a metal electrode. Chalcopyrite B and C are n-type chalcopyrite whose carrier concentrations were low ($-1.15\text{E}+18$ and $-4.27\text{E}+18\text{ cm}^{-3}$), indicating that this n-type chalcopyrite is not a degenerate semiconductor and should not act as a metal electrode (McMillan et al., 1982).

Leptospirillum ferriphilum strain (DQ343299) was provided by the Key Lab of Biohydrometallurgy of Ministry of Education, Central South University, Changsha, China. Microorganisms were cultured in the culture medium consisting of $(\text{NH}_4)_2\text{SO}_4$ (3.0 g/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 g/L), K_2HPO_4 (0.5 g/L), KCl (0.1 g/L), $\text{Ca}(\text{NO}_3)_2$ (0.01 g/L), and 44.7 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. Microorganisms grown to the exponential growth phase were harvested and centrifuged, and the obtained cells were inoculated into the 250-mL shake flasks containing 100 mL of sterilized culture medium and 1 g of chalcopyrite samples. Bioleaching experiments were carried out in shake flasks at a rotating speed of 170 rpm, temperature of 45°C , and pH 1.7. The cell density was determined in a blood counting chamber with a bright field microscope (CX31, Olympus Corporation, Tokyo, Japan). Sterile leaching experiments were conducted in basic culture medium without inoculating microorganisms, and Penbrite was used as the sterilizing agent. Ampicillin is a commonly used antibiotic for research and experimental use; its molecular formula is $\text{C}_{16}\text{H}_{25}\text{N}_3\text{O}_7\text{S}$ (CAS Number 7177-48-2). The culture medium was previously sterilized in an autoclave under 0.1 MPa and at 121°C for 30 min; the mineral samples were sterilized by ultraviolet light. During sterile leaching, the pH meter and potentiometer were all sterilized by ethyl alcohol, and sterility was checked by monitoring cell concentrations with a bright field microscope (CX31). Metal ions concentrations were determined by inductively coupled plasma-atomic emission spectrometer (America Baird Co. PS-6). The pH value was monitored with a pH meter (PHSJ-4A) and the redox potential was detected by an ORP meter (BPH-221).

Fig. 4.10 shows that copper extractions and redox potentials during sterile leaching increased steadily and slowly, and the leaching behavior of the three chalcopyrite samples was similar. However, the bioleaching behavior of the three chalcopyrite samples significantly differed. Final copper extractions of chalcopyrite A, B, and C were about 80%, 30%, and 10%. It was reported that the redox potential is the determining factor in chalcopyrite bioleaching. It should be maintained at an appropriate range (about 380–480 mV versus Ag/AgCl) to promote chalcopyrite dissolution (Córdoba et al., 2008; Gericke et al., 2010; Hiroyoshi et al., 2007; Petersen & Dixon, 2006; Third et al., 2000, 2002). Chalcopyrite dissolved extremely quickly when the redox potential was at the proposed appropriate range and almost stagnated when the redox potential was higher than 480 mV versus Ag/AgCl in this work. The redox potential of bioleaching for chalcopyrite A remained at an appropriate range for a longer time, which is a reason for the higher copper extraction. Fig. 4.10 shows

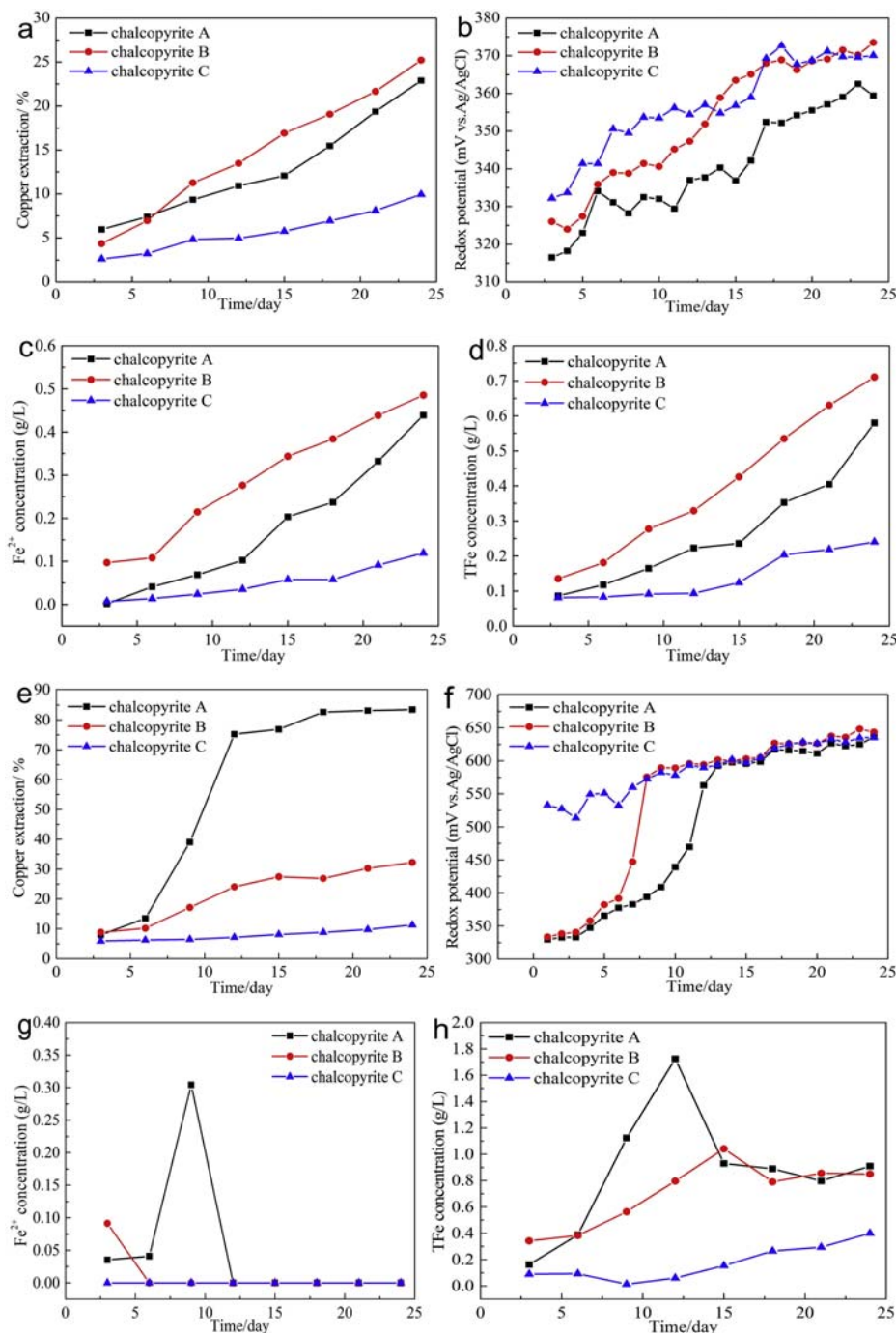


FIGURE 4.10 Sterile leaching of chalcopyrite samples: (A) Copper extraction, (B) Redox potential, (C) Fe^{2+} concentration, and (D) Total soluble Fe concentration. Bioleaching of chalcopyrite samples by *Leptospirillum ferriphilum*: (E) Copper extraction, (F) Redox potential, (G) Fe^{2+} concentration, and (H) Total Fe concentration. From Zhao, H., Huang, X., Wang, J., Li, Y., Liao, R., Wang, X., Qiu, X., Xiong, Y., Qin, W., & Qiu, G. (2017). Comparison of bioleaching and dissolution process of p-type and n-type chalcopyrite. *Minerals Engineering*, 109, 153–161. <https://doi.org/10.1016/j.mineng.2017.03.013>.

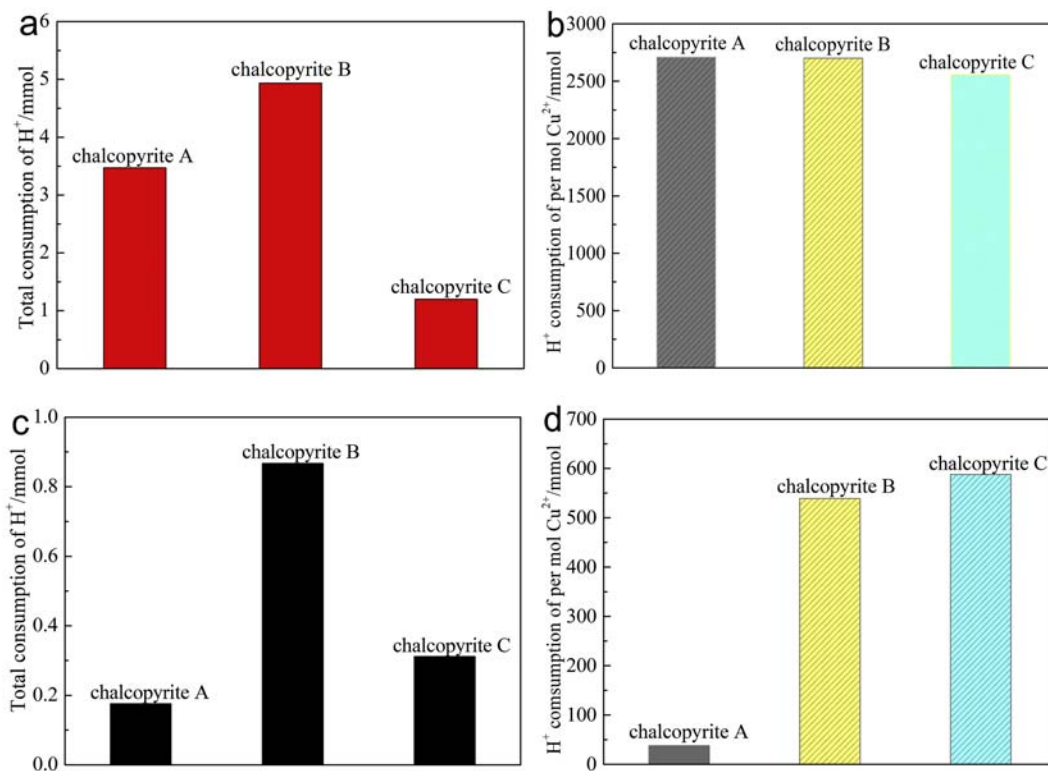


FIGURE 4.11 Sterile leaching of chalcopyrite samples: (A) Total H⁺ consumption. (B) H⁺ consumption of per gram Cu²⁺. Bioleaching of chalcopyrite samples by *Leptospirillum ferriphilum*. (C) Total H⁺ consumption. (D) H⁺ consumption of per gram Cu²⁺. From Zhao, H., Huang, X., Wang, J., Li, Y., Liao, R., Wang, X., Qiu, X., Xiong, Y., Qin, W., & Qiu, G. (2017). Comparison of bioleaching and dissolution process of p-type and n-type chalcopyrite. *Minerals Engineering*, 109, 153–161. <https://doi.org/10.1016/j.mineng.2017.03.013>.

that the dissolution behavior of iron in the sterile leaching of the three chalcopyrite samples was similar. Iron extraction by bioleaching chalcopyrite A increased remarkably faster than for the other two chalcopyrite samples. A high concentration of Fe²⁺ (between about the fifth and 12th days) may have caused the relatively low redox potential; it remained at the appropriate range for a longer time during the bioleaching of chalcopyrite A. The significant drop in total soluble iron at the later stage of bioleaching may be attributed to the precipitation of Fe³⁺, especially the formation of jarosite (Eq. 4.1). Fig. 4.11 shows that H⁺ consumption per gram of dissolved Cu²⁺ in the sterile leaching of the three chalcopyrite samples was similar. However, H⁺ consumption per gram of dissolved Cu²⁺ in the bioleaching of chalcopyrite A was significantly lower than that for the bioleaching of chalcopyrite B and C. The sterile leaching of chalcopyrite was mainly a nonoxidative dissolution process (Eq. 4.2), but bioleaching was an oxidative-reductive dissolution process (Li et al., 2013). Semiconducting properties cannot significantly affect nonoxidative dissolution, which is why the sterile leaching behavior of the three chalcopyrite samples was similar, including the metal (copper and iron) dissolution behavior and H⁺ consumption. However, semiconducting properties can remarkably affect oxidation-reduction reactions, resulting in different

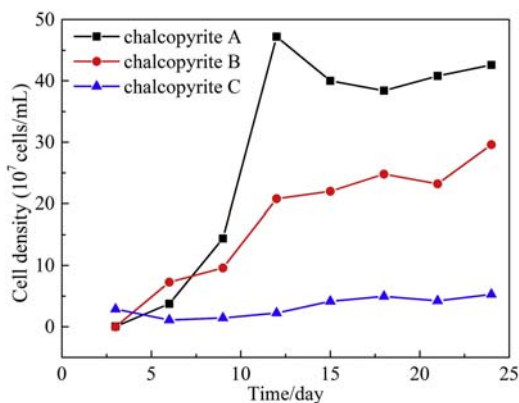
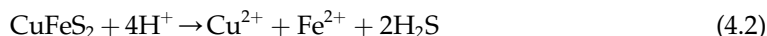


FIGURE 4.12 Growth curves of microorganisms during bioleaching of chalcopyrite samples. From Zhao, H., Huang, X., Wang, J., Li, Y., Liao, R., Wang, X., Qiu, X., Xiong, Y., Qin, W., & Qiu, G. (2017). Comparison of bioleaching and dissolution process of p-type and n-type chalcopyrite. *Minerals Engineering*, 109, 153–161. <https://doi.org/10.1016/j.mineng.2017.03.013>.

metal (copper and iron) dissolution behaviors and H^+ consumption. Fig. 4.12 indicates that *L. ferriphilum* in all of the bioleaching systems grew well and the growth of *L. ferriphilum* in the bioleaching system of chalcopyrite A was not inhibited. The redox potential in bioleaching systems mainly depends on the Fe^{3+}/Fe^{2+} ratio, and the main role of iron-oxidizing microorganisms (such as *L. ferriphilum*) in bioleaching is to oxidize Fe^{2+} to Fe^{3+} . Hence, the oxidation activity of microorganisms is an important factor affecting bioleaching processes by affecting the redox potential. It was reported that the oxidation activity of microorganisms should be controlled or inhibited to keep the redox potential in an appropriate range, accelerating chalcopyrite dissolution (Thirud et al., 2000, 2002).



4.2.2 Intermediate species analysis

A scanning electron microscope (JSM-6360 LV) equipped with an Energy Dispersive Spectrometer (EDS) was used to analyze the surface of solid samples. Fig. 4.13 indicates that the surface of all chalcopyrite samples (A, B, and C) was corroded to different degrees during bioleaching. However, chalcopyrite A during bioleaching was corroded most seriously, and the mineral surface was always in the form of a porous layer. Chalcopyrite samples leached by *L. ferriphilum* were analyzed by XPS. XPS was implemented with the model ESCALAB 250Xi, and the spectra were recorded at the constant pass energy of 20 and 0.1 eV/step with an Al K_{α} X-ray source. Thermo Avantage 5.52 software was used to fit the S 2p spectra, which were fitted using a 2:1 peak area ratio and 1.2 eV splitting for S 2p_{3/2} and S 2p_{1/2}. Binding energies were referred to the C 1s level at 284.8 eV. The background of spectra was achieved using the Shirley method and the S 2p spectra were fitted by the SGL function (Shirley, 1972). Sulfur species of polysulfide (S_n^{2-}), elemental sulfur (S^0), and sulfate (jarosite, SO_4^{2-}) were considered to be the possible compositions of a passivation layer. Hence, S 2p peaks were mainly analyzed in this work to investigate the intermediate species of the mineral

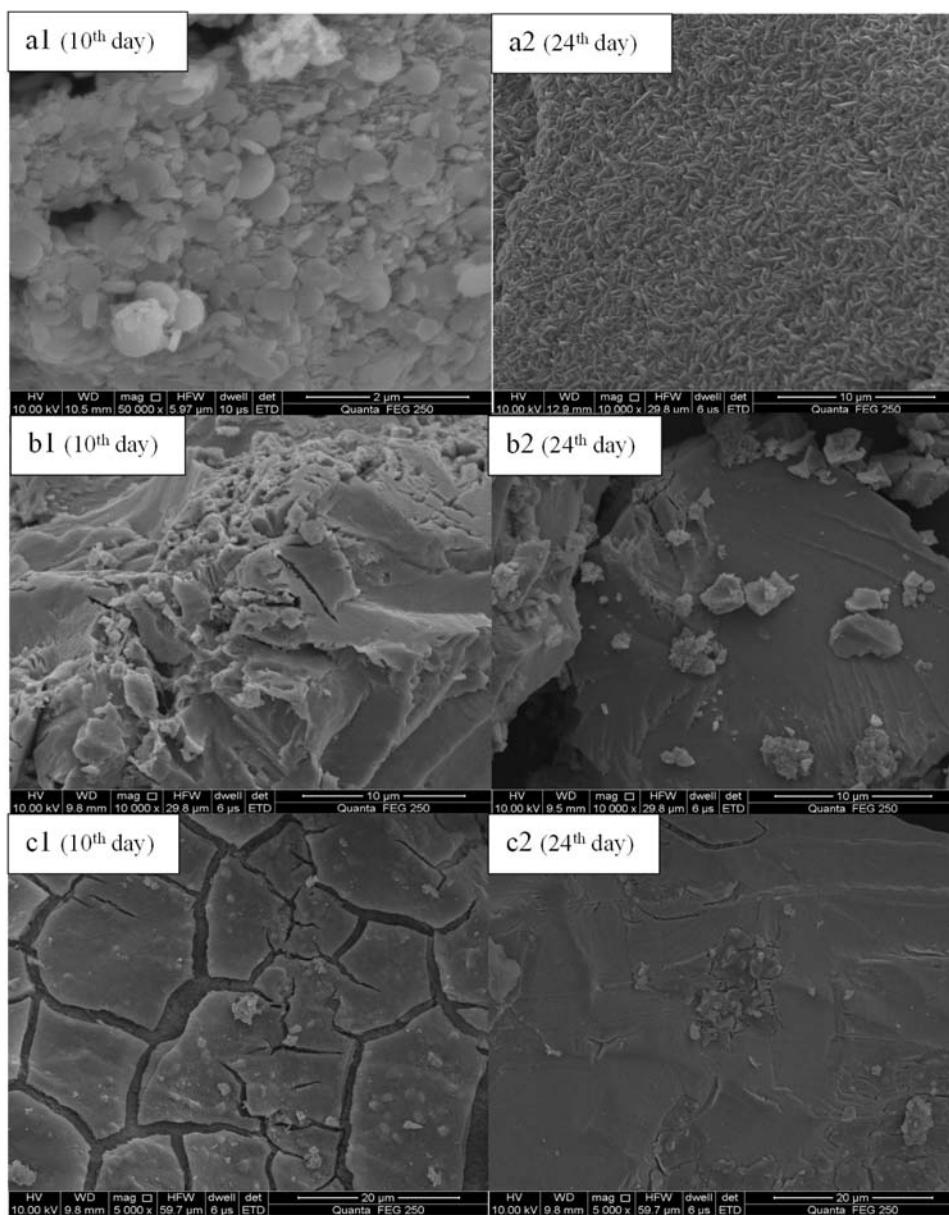


FIGURE 4.13 Scanning electron micrographs of chalcopyrite samples leached by *Leptospirillum ferriphilum* for various periods (10 and 24 days): (A) Chalcopyrite A; (B) Chalcopyrite B; and (C) Chalcopyrite C. (Magnification: a1 - 50000; a2, b1, b2 - 10000; c1, c2 - 5000). From Zhao, H., Huang, X., Wang, J., Li, Y., Liao, R., Wang, X., Qiu, X., Xiong, Y., Qin, W., & Qiu, G. (2017). Comparison of bioleaching and dissolution process of p-type and n-type chalcopyrite. *Minerals Engineering*, 109, 153–161. <https://doi.org/10.1016/j.mineng.2017.03.013>.

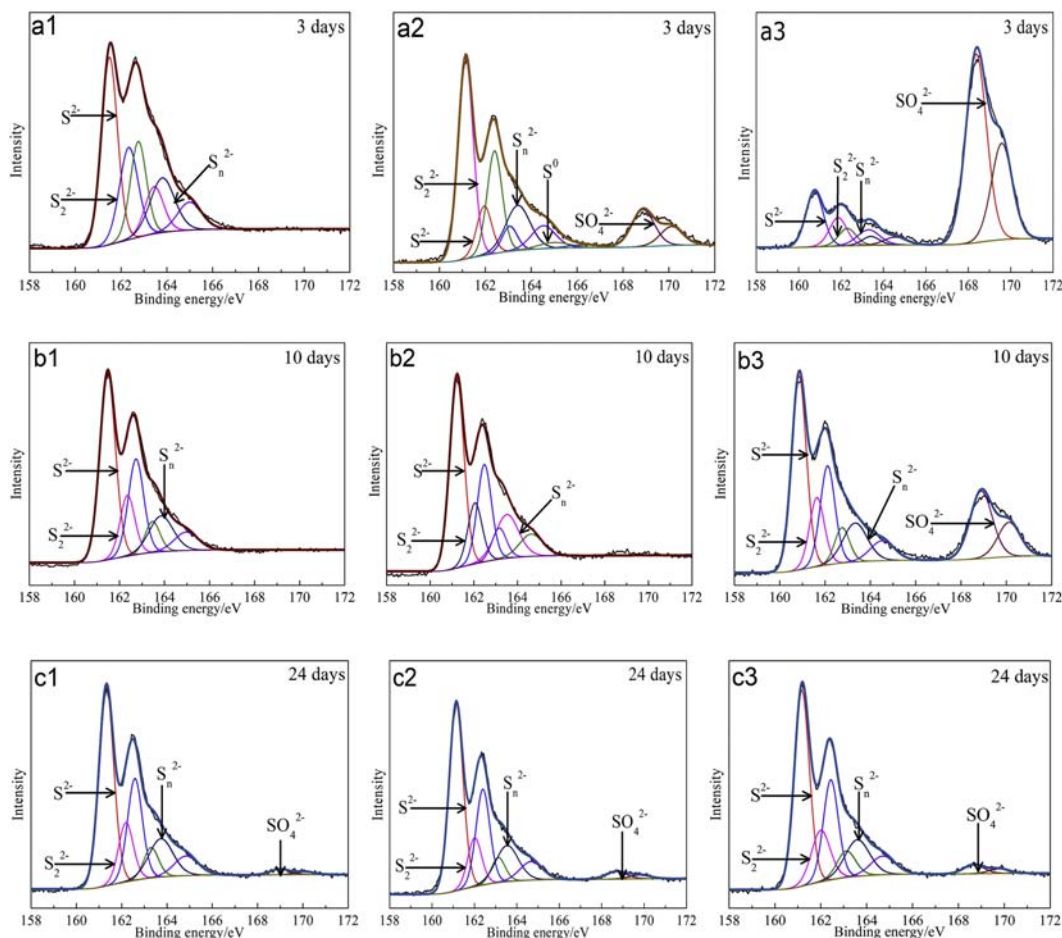


FIGURE 4.14 S 2p spectra of chalcopryite samples leached by *Leptospirillum ferriphilum* for various periods (3, 10, and 24 days): (A) Chalcopryite A; (B) Chalcopryite B; and (C) Chalcopryite C. From Zhao, H., Huang, X., Wang, J., Li, Y., Liao, R., Wang, X., Qiu, X., Xiong, Y., Qin, W., & Qiu, G. (2017). Comparison of bioleaching and dissolution process of *p*-type and *n*-type chalcopryite. *Minerals Engineering*, 109, 153–161. <https://doi.org/10.1016/j.mineng.2017.03.013>.

surface during bioleaching of chalcopryite samples. S 2p_{3/2} peaks were fitted, and the corresponding binding energy and full width at half maximum values were compared with references (Acres et al, 2010a, 2010b, 2011; Buckley & Woods, 1984; Ghahremaninezhad et al., 2013; Harmer et al., 2006; Klauber et al., 2001; Smart et al., 1999). S 2p_{3/2} peaks of chalcopryite A leached by *L. ferriphilum* for different days (3, 10, and 24 days) were fitted (Fig. 4.14). Mono-sulfide (S²⁻), disulfide (S₂²⁻), and polysulfide were the main sulfur species of chalcopryite A leached for 3 days; S²⁻, S₂²⁻, S⁰, S_n²⁻, and SO₄²⁻ were the main sulfur species on the 10th day; and S²⁻, S₂²⁻, S_n²⁻, and SO₄²⁻ were the main intermediate species of chalcopryite A leached for 24 days. For the bioleaching of chalcopryite B, S²⁻, S₂²⁻, and S_n²⁻ were the main sulfur species on the third and 10th days, and SO₄²⁻ occurred on the mineral surface leached for 24 days. S²⁻, S₂²⁻, S_n²⁻, and SO₄²⁻ were the main sulfur species during the bioleaching of chalcopryite C. Thus, the intermediate species for the bioleaching of chalcopryite A, B, and C were similar.

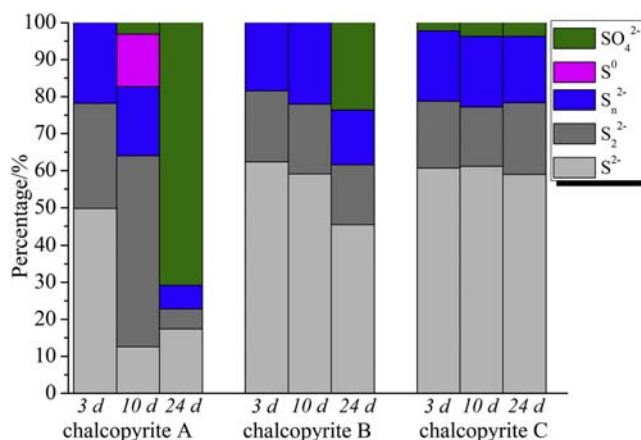


FIGURE 4.15 Distribution of sulfur species on the surface of leached chalcopyrite samples. From Zhao, H., Huang, X., Wang, J., Li, Y., Liao, R., Wang, X., Qiu, X., Xiong, Y., Qin, W., & Qiu, G. (2017). Comparison of bioleaching and dissolution process of p-type and n-type chalcopyrite. *Minerals Engineering*, 109, 153–161. <https://doi.org/10.1016/j.mineng.2017.03.013>.

The distribution of sulfur species on the surface of leached chalcopyrite samples is shown in Fig. 4.15. Polysulfide existed throughout bioleaching and was considered to be the main composition of passivation layer in previous studies. However, a high percentage of polysulfide did not hinder the dissolution of chalcopyrite A in this work.

4.2.3 Electrochemical analysis

An electrochemical analysis was carried out to interpret the differences of dissolution processes further. Electrochemical tests were implemented by a conventional three-electrode system (Princeton Model 283 Potentiostat, EG&G, Princeton Applied Research). All potential values mentioned refer to the Ag/AgCl electrode (versus Ag/AgCl) in this work. The Tafel

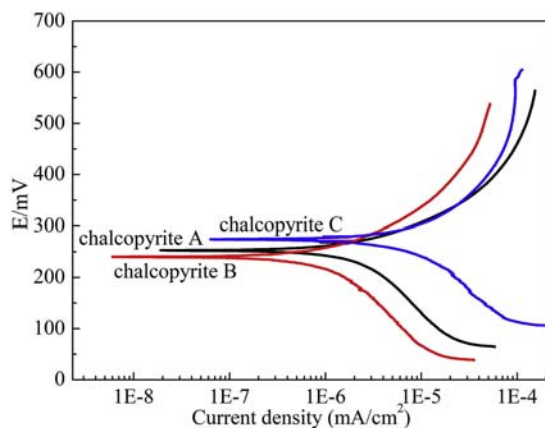


FIGURE 4.16 Tafel curves of chalcopyrite samples in basic culture medium. From Zhao, H., Huang, X., Wang, J., Li, Y., Liao, R., Wang, X., Qiu, X., Xiong, Y., Qin, W., & Qiu, G. (2017). Comparison of bioleaching and dissolution process of p-type and n-type chalcopyrite. *Minerals Engineering*, 109, 153–161. <https://doi.org/10.1016/j.mineng.2017.03.013>.

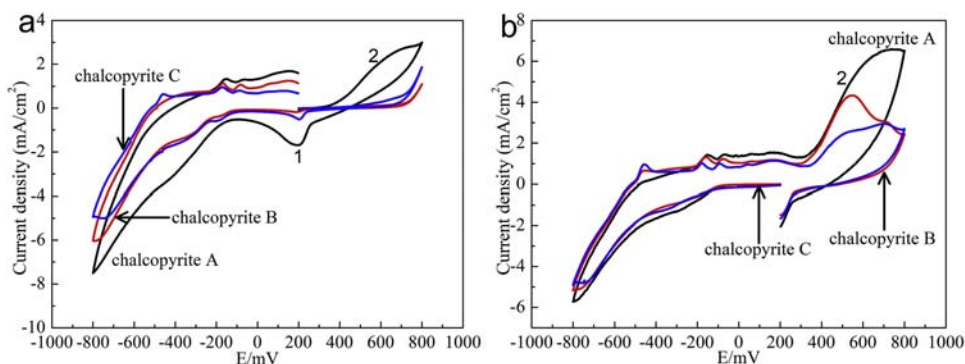


FIGURE 4.17 Cyclic voltammograms of chalcopyrite samples in basic culture medium: (A) Positive scan route; (B) Negative scan route. From Zhao, H., Huang, X., Wang, J., Li, Y., Liao, R., Wang, X., Qiu, X., Xiong, Y., Qin, W., & Qiu, G. (2017). Comparison of bioleaching and dissolution process of *p*-type and *n*-type chalcopyrite. *Minerals Engineering*, 109, 153–161. <https://doi.org/10.1016/j.mineng.2017.03.013>.

test (Fig. 4.16) shows that corrosion potentials (E_{corr}) of chalcopyrite A, B, and C were similar (252, 240, 274 mV versus Ag/AgCl). Fig. 4.17 presents cyclic voltammograms of chalcopyrite samples in the positive scan route and negative scan route. The electrochemical dissolution processes of the three chalcopyrite samples were similar. Peak 1 was considered to be the reduction of chalcopyrite to Cu_2S in the presence of Cu^{2+} , as shown in Eq. 4.3 (Liang et al., 2011; Warren et al., 1985; Zhao et al., 2013). Its peak potential of chalcopyrite A shifted negatively and the current density was higher, indicating that chalcopyrite A was more easily transformed to Cu_2S . Peak 2 was assigned to the decomposition of chalcopyrite to metal-deficient polysulfide, as shown in Eq. 4.4 (Arce & González, 2002; Liang et al., 2011; Zhao et al., 2013). The current density of peak 2 of chalcopyrite A was significantly higher, indicating that the formed polysulfide has good electrochemical activity and would not hinder the charge transfer process.

Potentiostatic polarization experiments (Fig. 4.18) show that the three chalcopyrite samples can be directly oxidized when the redox potential was higher than 0.3 V versus Ag/AgCl. The

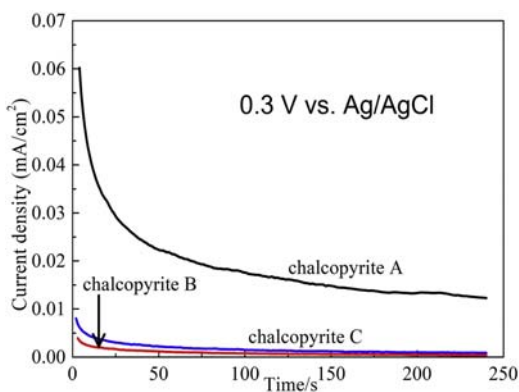


FIGURE 4.18 Potentiostatic polarization of chalcopyrite samples in basic culture medium at 0.3 V. From Zhao, H., Huang, X., Wang, J., Li, Y., Liao, R., Wang, X., Qiu, X., Xiong, Y., Qin, W., & Qiu, G. (2017). Comparison of bioleaching and dissolution process of *p*-type and *n*-type chalcopyrite. *Minerals Engineering*, 109, 153–161. <https://doi.org/10.1016/j.mineng.2017.03.013>.

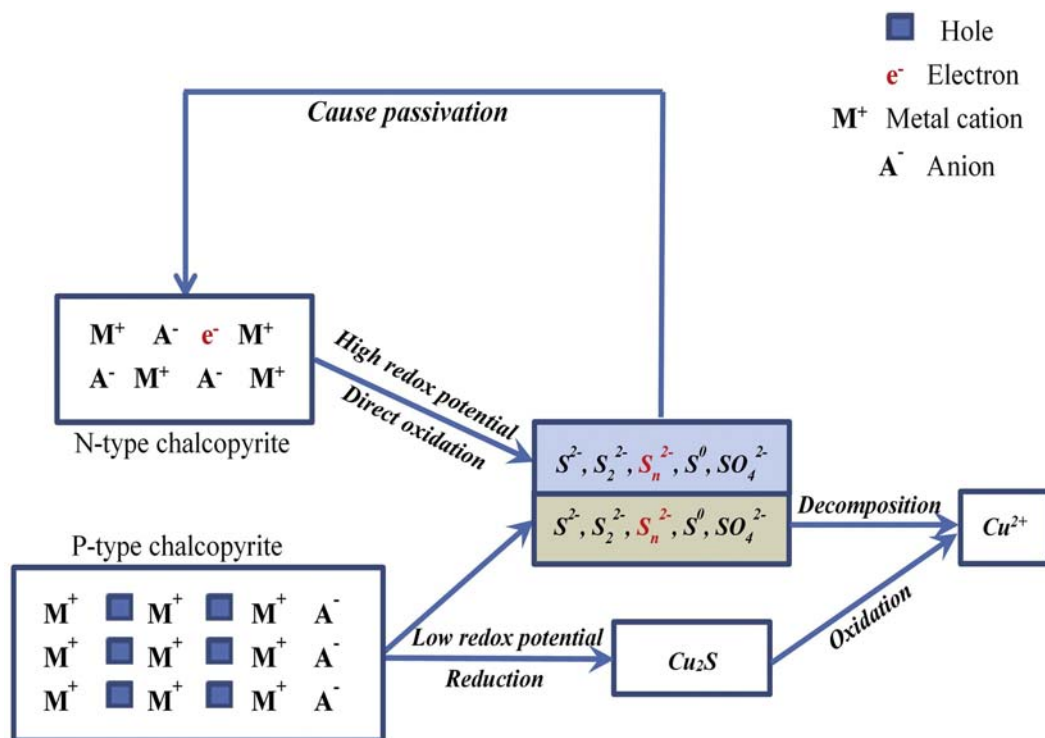


FIGURE 4.19 Proposed model of dissolution mechanism for different chalcopyrite samples. From Zhao, H., Huang, X., Wang, J., Li, Y., Liao, R., Wang, X., Qiu, X., Xiong, Y., Qin, W., & Qiu, G. (2017). Comparison of bioleaching and dissolution process of p-type and n-type chalcopyrite. *Minerals Engineering*, 109, 153–161. <https://doi.org/10.1016/j.mineng.2017.03.013>.

conductivity of chalcopyrite A was significantly higher than that of the other two in the oxidation process. Hence, higher values of conductivity and oxidation-reduction rate also contributed to the high bioleaching efficiency of chalcopyrite A.

Based on these results, a model of dissolution mechanism of different chalcopyrite samples was proposed in Fig. 4.19.

4.3 Brief overview of effects of mineralogical properties

The dissolution process and passivation mechanism of chalcopyrite vary with the mineralogical properties and physicochemical properties of chalcopyrite. Therefore, the effects of the physicochemical properties of chalcopyrite on the dissolution and passivation mechanism of chalcopyrite bioleaching need to be interpreted.

4.3.1 Semiconductor property

With a bandgap of 0.4–0.6 eV, chalcopyrite is widely regarded as a semiconductor (Crundwell, 2003; Teranishi & Sato, 1975; Tributsch & Bennett, 1981). Semiconductor properties of chalcopyrite determine the electron transfer rate, which affects its oxidation-reduction

rate. The Fermi level of chalcopyrite is just 0.15 eV higher than the top of the valence band, and it is mainly composed of Cu 3d, 0.3 eV lower than the bottom of the conduction band composed of Fe 3d (Hamajima et al., 1981). However, Edelbro et al. (2003) claimed that chalcopyrite should be regarded as a conductor because there was no band crossing at the Fermi level.

Hiskey (1993) stated that the semiconductor properties and passive film of chalcopyrite were both responsible for the different dissolution rates under different conditions of redox potential. Crundwell (2015) proposed a semiconductor theory in which the dissolution of chalcopyrite was influenced by the semiconductor properties of minerals, not by passive film. The anodic dissolution process of chalcopyrite was at a potential of 540–640 mV versus SHE, and there was a barrier region where the current was limited by the electron consumption of the space charge layer; therefore, no reaction could provide the required electrons, so it was difficult to leach chalcopyrite. In solution, the electric double-layer structure was formed at the interface between the chalcopyrite and the solution. Charges of the electric double layer could be divided into three categories: space-charge, Helmholtz, and Gouy-Chapman. For a chalcopyrite semiconductor, charges in the applied potential mainly affected the state of the space-charge layer and then influenced the energy levels of the solid side electrons in the space-charge layer. Studies showed that when the applied potential increased to more than 0.94 V (versus SHE), mobile holes accumulated on the chalcopyrite surface. These holes were beneficial to the flow of anodic currents. Hence, the reason for the low dissolution rate of chalcopyrite was the limited rate of holes supplied to the surface. A schematic diagram of the chalcopyrite band structure is provided in Fig. 4.20 (Congren et al., 2018; Crundwell, 2015). The energy median (Eredox, unit-eV) between the occupied and unoccupied states is directly related to the oxidation/reduction potential (unit-V) of the redox couple (Roy, 1978), and the Eredox of different redox couples were calculated from the ORPs (Frignani et al., 2001). The Eredox of the $\text{Fe}^{3+}/\text{Fe}^{2+}$ couple is always situated above the valence band, although the ratio of $\text{Fe}^{3+}/\text{Fe}^{2+}$ is high; therefore, it is difficult for Fe^{3+} to capture electrons from chalcopyrite surface, which causes the low dissolution kinetics of chalcopyrite. When the ratio of $\text{Fe}^{3+}/\text{Fe}^{2+}$ is low, the Eredox is located above the Fermi energy. Hence, chalcopyrite can capture electrons from the $\text{Fe}^{3+}/\text{Fe}^{2+}$ couple and is degraded to intermediate species such as Cu_2S , causing the high dissolution kinetics of chalcopyrite. The Eredox of the redox couple of $\text{O}_2/\text{H}_2\text{O}$ is always situated below the Fermi energy and in the range of the valence band. Thus, O_2 can easily capture electrons from the chalcopyrite surface, resulting in a high dissolution rate of chalcopyrite. Nevertheless, the solubility of dissolved O_2 in water is extremely low at normal pressure, which is why high pressure is essential for oxygen-rich leaching.

Doping can significantly change the semiconductor properties of chalcopyrite, causing differences in the dissolution process. In nature, chalcopyrite can be doped by different elements during the mineralization stage, resulting in different semiconductor types and properties. This may be an important reason for the different oxidative dissolution results of chalcopyrite samples from different regions (McMillan et al., 1982). In addition, the theory of chalcopyrite doping can be used to develop techniques to promote chalcopyrite bioleaching. For example, we can try to find catalysts for chalcopyrite bioleaching based on the doped elements, or to convert the types of chalcopyrite semiconductor. Inspiration may be achieved from the research area of ternary chalcopyrite semiconductors.

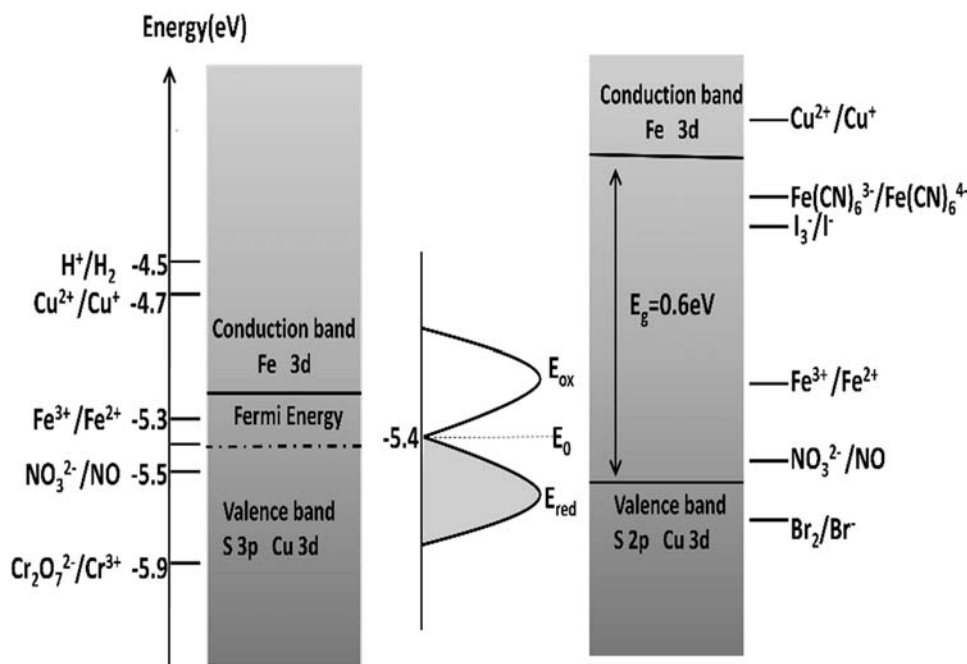


FIGURE 4.20 Schematic diagram of chalcopyrite band structure (Crundwell, 2015; Congren et al., 2018). From Zhao, H., Zhang, Y., Zhang, X., Qian, L., Sun, M., Yang, Y., Zhang, Y., Wang, J., Kim, H., & Qiu, G. (2019). The dissolution and passivation mechanism of chalcopyrite in bioleaching: An overview. *Minerals Engineering*, 136, 140–154. <https://doi.org/10.1016/j.mineng.2019.03.014>.

Ternary chalcopyrite semiconductors, which can be clarified into I-III-VI₂ and II-IV-V₂ compounds, have attracted a lot of attention for their potential applications in detectors, solar cells, and nonlinear optical devices. In addition, many studies have investigated the photovoltaic applications of the I-III-VI₂ family. I-III-VI₂ chalcopyrite compounds can be represented by the formula ABC₂ (A = Ag, Cu; B = Ga, In; and C = S, Se, Te), which all exhibit the chalcopyrite crystal structure and are crystallized in the space group *I*-42d with four formula units in each unit cell (Chichibu et al., 1997; Rashkeev & Lambrecht, 2001; Xue et al., 2000). The main I-III-VI₂ chalcopyrite semiconductors and their parameters are provided in Table 4.4 (Rashkeev & Lambrecht, 2001; Xue et al., 2000). Principally, n-type and p-type semiconductor use electrons and holes as their carriers, respectively. The 3d orbit is important to the magnetic, electrical, and optical properties of both n-type and p-type chalcopyrite. The properties of chalcopyrite can be changed by doping (Teranishi & Sato, 1975). The two types of semiconductors can also be converted to each other by doping (Albor Aguilera et al., 2007; Lany et al., 2005; Sieben-tritt et al., 2010). High pressure (12.5–14.8 GPa) has proved to be a useful technique in the conversion between n and p type (Kumar et al., 2000).

4.3.2 Phase

At 50 and 190K, chalcopyrite has magnetic phases, so it has magnetism at low temperatures. From the magnetic phase temperature to 823K, chalcopyrite has antiferromagnetism (Rais et al., 2000; Woolley et al., 1996). At room temperature, the magnetic torque of Fe

TABLE 4.4 Parameters of I-III-VI₂-type chalcopyrite semiconductors (Rashkeev & Lambrecht, 2001; Xue et al., 2000).

Compound	Bond	Bond length d ^u (Å)	Bond covalency f ^u _c	Bond charge q ^u /e	Eg ^{ldaa}	Eg ^{exptb} /eV
CuGaS ₂	Cu–S	2.380	0.359	0.731	0.92	2.43
	Ga–S	2.224	0.289	0.862		
CuInS ₂	Cu–S	2.288	0.379	0.746	0.01	1.53
	In–S	2.517	0.282	0.755		
CuGaSe ₂	Cu–Se	2.417	0.458	0.659	0.20	1.68
	Ga–Se	2.417	0.281	0.777		
CuInSe ₂	Cu–Se	2.424	0.318	0.728	0.01	1.04
	In–Se	2.598	0.279	0.714		
CuGaTe ₂	Cu–Te	2.590	0.307	0.682	0.43	1–1.24
	Ga–Te	2.590	0.278	0.720		
CuInTe ₂	Cu–Te	2.585	0.290	0.703	0.18	1.06
	In–Te	2.763	0.277	0.665		
AgGaS ₂	Ag–S	2.605	0.214	0.870	1.02	2.64
	Ga–S	2.235	0.294	0.836		
AgInS ₂	Ag–S	2.505	0.320	0.716	0.35	1.87
	In–S	2.505	0.280	0.765		
AgGaSe ₂	Ag–Se	2.601	0.292	0.693	0.17	1.80
	Ga–Se	2.416	0.284	0.769		
AgInSe ₂	Ag–Se	2.610	0.278	0.713	0.10	1.24
	In–Se	2.610	0.278	0.714		
AgGaTe ₂	Ag–Te	2.714	0.288	0.662	0.17	1.32
	Ga–Te	2.640	0.278	0.705		
AgInTe ₂	Ag–Te	2.756	0.282	0.660	0.21	0.95
	In–Te	2.756	0.276	0.669		

and Cu is 3.85 and 0.2 m_B, respectively (Donnay et al., 1917; Jones, 2006). At the conversion temperature (T_c), chalcopyrite can be converted into a disorderly sphalerite structure (Su et al., 2000). Furthermore, it was reported that chalcopyrite has three kinds of polymorphs: a-chalcopyrite (tetrahedral structure), b-chalcopyrite, and g-chalcopyrite. They exhibit different dissolution kinetics in bioleaching (Hall and Stewart, 1973; Zhang, 2011). In an inert atmosphere, when the temperature is raised to 673K, the sulfur element in the a-chalcopyrite begins to volatilize, and the b-chalcopyrite of cubic structure is produced with an increase in lattice parameters. When the temperature reaches 823K, g-chalcopyrite of cubic structure is

produced along with an increase in lattice parameters, but this phase is not stable, so g-chalcopyrite is gradually transformed into b-chalcopyrite as the temperature decreases (Hall and Stewart, 1973; Zhang, 2011). This indicates that chalcopyrite in the earth may exist in different kinds of polymorphs. In addition, techniques such as roasting may be considered to convert chalcopyrite phases and improve bioleaching efficiency.

4.3.3 Anisotropic crystal surface

Usually, the surface of chalcopyrite appears as a shell shape owing to its imperfect cleavage, so it often shows anisotropy after being crushed. It is widely believed that cleavage planes (100), (001), (111), (101), (012), (110), and (112) appear in chalcopyrite, and there are usually three sulfides on the fresh surface, including monosulfide (S^{2-}), disulfide (S_2^{2-}), and polysulfide (S_n^{2-}) (De Oliveira & Duarte, 2010; Harmer et al., 2004; Von Oertzen et al., 2006; Zhang et al., 2019). In addition, CuO, Cu₂O, FeOOH, and sulfate (SO_4^{2-}) have been detected on the chalcopyrite surface under air conditions (Buckley & Woods, 1984; Goh et al., 2006; Todd et al., 2003; Zhang et al., 2019). Biological activities of microorganisms can significantly alter the surface properties of chalcopyrite, including the surface structure, wettability, and electric conductivity (Chandraprabha et al., 2004; Chen et al., 2008; Devasia et al., 1993). Oliveira et al. (De Oliveira & Duarte, 2010; De Oliveira et al., 2012) used DFT calculations to study the surface reconstruction of chalcopyrite and suggested that different passivation mechanisms may occur depending on the crystal orientation. De Lima et al. (2011, 2012) further studied the adsorption mechanisms of water and sulfuric and hydrochloric acids on the (001) chalcopyrite surface through DFT calculations. Those studies provided insights into the effects of anisotropic crystal surface characteristics on the dissolution and passivation mechanism of chalcopyrite. However, related progress is still rarely reported, mainly owing to an insufficiency of precise techniques and the difficulty of preparing chalcopyrite samples with different exposure surfaces.

Different dissolution and passivation mechanisms may take place depending on the crystal orientation. Hence, selective crushing or milling may be used to control the proportions of different cleavage planes and enhance the bioleaching efficiency of chalcopyrite. For example, Li and Gao (2018) regulated the degree of exposure of crystal surfaces of fluorite by using different grinding media. They found that the shape seemed to be directly related to the proportion of crystal surfaces exposed in mineral particles. However, related work for chalcopyrite has rarely been reported.

A model is provided to interpret the dissolution and passivation mechanism of chalcopyrite in bioleaching according to existing publications (Fig. 4.21).

4.4 Summary

Monosulfide (S^{2-}), disulfide (S_2^{2-}), polysulfide (S_n^{2-}), elemental sulfur (S^0), and sulfate (SO_4^{2-}) were the main intermediate species on the surface of chalcopyrite during bioleaching by moderately thermophilic microorganisms. According to the results of electrochemical studies, the solubility of chalcopyrite under different potential conditions was proposed. When the potential was 250–650 mV, chalcopyrite was passivated and 700 mV might reach

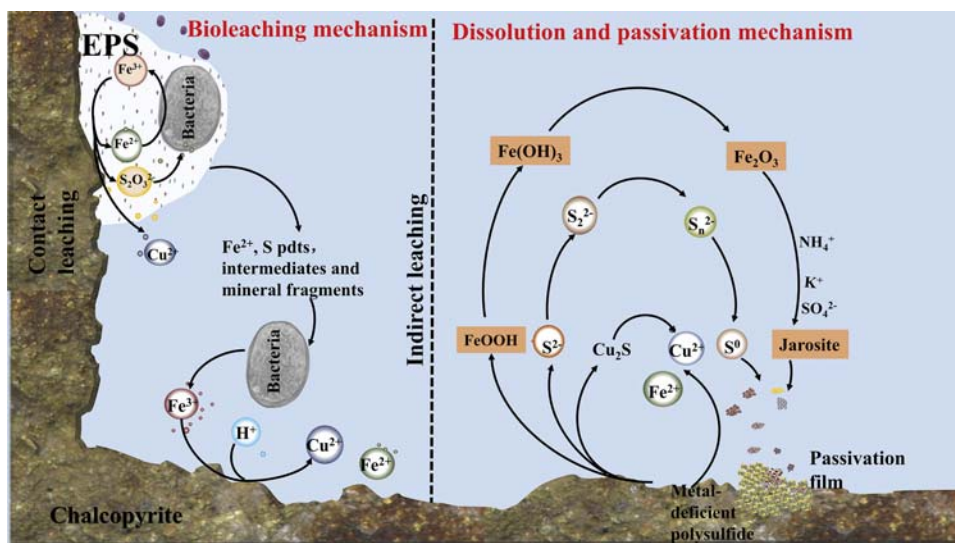


FIGURE 4.21 Passivation and dissolution mechanism of chalcopyrite in bioleaching. The left part of Fig. 4.21 shows bacterial attachment to chalcopyrite. The right part of Fig. 4.21 shows the dissolution and passivation mechanisms of chalcopyrite in bioleaching. (1) Chalcopyrite degrades into Cu_2S , which accelerates the dissolution rate. (2) Chalcopyrite releases Fe^{2+} and Cu^{2+} directly, resulting in the formation of metal-deficient polysulfide. (3) Elemental sulfur film is formed. (4) Jarosite is precipitated on the surface of chalcopyrite. Paths 2, 3, and 4 may cause the passivation of chalcopyrite. From Zhao, H., Zhang, Y., Zhang, X., Qian, L., Sun, M., Yang, Y., Zhang, Y., Wang, J., Kim, H., & Qiu, G. (2019). The dissolution and passivation mechanism of chalcopyrite in bioleaching: An overview. *Minerals Engineering*, 136, 140–154. <https://doi.org/10.1016/j.mineng.2019.03.014>.

the transpassivation potential. However, there is a special interval (around 450 mV) in the passivation range of 250–650 mV in which chalcopyrite was dissolved relatively quickly.

Remarkably higher copper extraction and low acid consumption of p-type chalcopyrite with high carrier concentrations could be achieved during bioleaching by *L. ferrophilum* compared with n-type chalcopyrite at low carrier concentrations. Intermediate species and electrochemical dissolution processes were similar in bioleaching of n-type and p-type chalcopyrite. P-type chalcopyrite was more easily corroded than n-type chalcopyrite, and the formed polysulfide (S_n^{2-}) did not hinder the further dissolution of p-type chalcopyrite owing to its good electrochemical activity. Higher values of conductivity and the oxidation-reduction rate also contributed to the high bioleaching efficiency of p-type chalcopyrite.

The dissolution and passivation mechanisms of chalcopyrite bioleaching are closely related to its semiconductor properties. A different point of view believes that the semiconducting properties of chalcopyrite, instead of the passivation layer, cause the low dissolution rate of chalcopyrite. Different dissolution and passivation mechanisms may occur depending on the crystal orientation.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (51704331), Young Elite Scientists Sponsorship Program by CAST (2017QNRC001), National Basic Research Program of China (Grant No. 2010CB630905), and High-Tech Research and Development Program of China (Grant No. 2012AA061501).

References

- Acres, R. G., Harmer, S. L., & Beattie, D. A. (2010a). Synchrotron XPS, NEXAFS, and ToF-SIMS studies of solution exposed chalcopyrite and heterogeneous chalcopyrite with pyrite. *Minerals Engineering*, 23(11–13), 928–936. <https://doi.org/10.1016/j.mineng.2010.03.007>.
- Acres, R. G., Harmer, S. L., & Beattie, D. A. (2010b). Synchrotron XPS studies of solution exposed chalcopyrite, bornite, and heterogeneous chalcopyrite with bornite. *International Journal of Mineral Processing*, 94(1–2), 43–51. <https://doi.org/10.1016/j.minpro.2009.11.006>.
- Acres, R. G., Harmer, S. L., Shui, H. W., Chen, C. H., & Beattie, D. A. (2011). Synchrotron scanning photoemission microscopy of homogeneous and heterogeneous metal sulfide minerals. *Journal of Synchrotron Radiation*, 18(4), 649–657. <https://doi.org/10.1107/S0909049511007175>.
- Ahmadi, A., Schaffie, M., Manafi, Z., & Ranjbar, M. (2010). Electrochemical bioleaching of high grade chalcopyrite flotation concentrates in a stirred bioreactor. *Hydrometallurgy*, 104(1), 99–105. <https://doi.org/10.1016/j.hydromet.2010.05.001>.
- Albor Aguilera, M. L., Aguilar Hernandez, J. R., Gonzalez Trujillo, M. A., & Ortega lopez, M. (2007). Photoluminescence studies of p-type chalcopyrite AgInS₂:Sn. *Solar Energy Materials and Solar Cells*, 1483–1487. <https://doi.org/10.1016/j.solmat.2007.05.006>.
- Arce, E. M., & González, I. (2002). A comparative study of electrochemical behavior of chalcopyrite, chalcocite and bornite in sulfuric acid solution. *International Journal of Mineral Processing*, 67(1–4), 17–28. [https://doi.org/10.1016/S0301-7516\(02\)00003-0](https://doi.org/10.1016/S0301-7516(02)00003-0).
- Bevilaqua, D., Diéz-Perez, I., Fugivara, C. S., Sanz, F., Benedetti, A. V., & Garcia, O. (2004). Oxidative dissolution of chalcopyrite by *Acidithiobacillus ferrooxidans* analyzed by electrochemical impedance spectroscopy and atomic force microscopy. *Bioelectrochemistry*, 64(1), 79–84. <https://doi.org/10.1016/j.bioelechem.2004.01.006>.
- Blight, K. R., Candy, R. M., & Ralph, D. E. (2009). The preferential oxidation of orthorhombic sulfur during batch culture. *Hydrometallurgy*, 99(1–2), 100–104. <https://doi.org/10.1016/j.hydromet.2009.05.013>.
- Brion, D. (1980). Etude par spectroscopie de photoelectrons de la degradation superficielle de FeS₂, CuFeS₂, ZnS et PbS a l'air et dans l'eau. *Applications of Surface Science*, 5(2), 133–152. [https://doi.org/10.1016/0378-5963\(80\)90148-8](https://doi.org/10.1016/0378-5963(80)90148-8).
- Buckley, A. N., & Woods, R. (1984). An X-Ray photoelectron spectroscopic study of the oxidation of chalcopyrite. *Australian Journal of Chemistry*, 37(12), 2403–2413. <https://doi.org/10.1071/CH9842403>.
- Casas, J., Lienqueo, M., Cubillos, F., & Herrera, L. (2000). *Modelacion cinetica de la precipitacion de hierro como jarosita en soluciones lixiviantes utilizando la bacteria Thiobacillus, & ferroxidans*. Universidad de Santiago.
- Chandraprabha, M. N., Natarajan, K. A., & Modak, J. M. (2004). Selective separation of pyrite and chalcopyrite by biomodulation. *Colloids and Surfaces B: Biointerfaces*, 37(3–4), 93–100. <https://doi.org/10.1016/j.colsurfb.2004.06.011>.
- Chen, M. L., Zhang, L., Gu, G. H., Hu, Y. H., & Su, L. J. (2008). Effects of microorganisms on surface properties of chalcopyrite and bioleaching. *Transactions of Nonferrous Metals Society of China*, 18(6), 1421–1426. [https://doi.org/10.1016/S1003-6326\(09\)60019-4](https://doi.org/10.1016/S1003-6326(09)60019-4).
- Chichibu, S., Shirakata, S., Isomura, S., & Nakanishi, H. (1997). Visible and ultraviolet photoluminescence from Cu-III-VI₂ chalcopyrite semiconductors grown by metalorganic vapor phase epitaxy. *Japanese Journal of Applied Physics. Part 1: Regular Papers and Short Notes and Review Papers*, 36(3), 1703–1714. <https://doi.org/10.1143/jjap.36.1703>.
- Chusuei, C. C., Brookshier, M. A., & Goodman, D. W. (1999). Correlation of relative X-ray photoelectron spectroscopy shake-up intensity with CuO particle size. *Langmuir*, 15(8), 2806–2808. <https://doi.org/10.1021/la9815446>.
- Congren, Y., Wenqing, Q., Hongbo, Z., Jun, W., & Xingjie, W. (2018). Mixed potential plays a key role in leaching of chalcopyrite: Experimental and theoretical analysis. *Industrial and Engineering Chemistry Research*, 1733–1744. <https://doi.org/10.1021/acs.iecr.7b02051>.
- Córdoba, E. M., Muñoz, J. A., Blázquez, M. L., González, F., & Ballester, A. (2008). Leaching of chalcopyrite with ferric ion. Part IV: The role of redox potential in the presence of mesophilic and thermophilic bacteria. *Hydrometallurgy*, 93(3–4), 106–115. <https://doi.org/10.1016/j.hydromet.2007.11.005>.
- Crundwell, F. K. (2003). How do bacteria interact with minerals? *Hydrometallurgy*, 71(1–2), 75–81. [https://doi.org/10.1016/S0304-386X\(03\)00175-0](https://doi.org/10.1016/S0304-386X(03)00175-0).
- Crundwell, F. K. (2015). The semiconductor mechanism of dissolution and the pseudo-passivation of chalcopyrite. *Canadian Metallurgical Quarterly*, 1.
- David. (2002). *Hall effect in semiconductors*.

- De Lima, G. F., De Oliveira, C., De Abreu, H. A., & Duarte, H. A. (2011). Water adsorption on the reconstructed (001) chalcopyrite surfaces. *Journal of Physical Chemistry C*, 115(21), 10709–10717. <https://doi.org/10.1021/jp201106e>.
- De Lima, G. F., De Oliveira, C., De Abreu, H. A., & Duarte, H. A. (2012). Sulfuric and hydrochloric acid adsorption on the reconstructed sulfur terminated (001) chalcopyrite surface. *International Journal of Quantum Chemistry*, 112(19), 3216–3222. <https://doi.org/10.1002/qua.24145>.
- De Oliveira, C., De Lima, G. F., De Abreu, H. A., & Duarte, H. A. (2012). Reconstruction of the chalcopyrite surfaces-A DFT study. *Journal of Physical Chemistry C*, 116(10), 6357–6366. <https://doi.org/10.1021/jp300713z>.
- De Oliveira, C., & Duarte, H. A. (2010). Disulphide and metal sulphide formation on the reconstructed (0 0 1) surface of chalcopyrite: A DFT study. *Applied Surface Science*, 257(4), 1319–1324. <https://doi.org/10.1016/j.apsusc.2010.08.059>.
- Devasia, P., Natarajan, K. A., Sathyanarayana, D. N., & Rao, G. R. (1993). Surface chemistry of Thiobacillus ferrooxidans relevant to adhesion on mineral surfaces. *Applied and Environmental Microbiology*, 59(12), 4051–4055. <https://doi.org/10.1128/aem.59.12.4051-4055.1993>.
- Donnay, G., Corliss, L., Donnay, J., Elliott, N., & Hastings, J. (1917). Symmetry of magnetic structures: Magnetic structure of chalcopyrite. *Physical Review*, 112.
- Dutrizac, J. E. (1989). Elemental sulphur formation during the ferric sulphate leaching of chalcopyrite. *Canadian Metallurgical Quarterly*, 28(4), 337–344. <https://doi.org/10.1179/cm.1989.28.4.337>.
- Edelbro, R., Sandström, A., & Paul, J. (2003). Full potential calculations on the electron bandstructures of Sphalerite, Pyrite and Chalcopyrite. *Applied Surface Science*, 206(1–4), 300–313. [https://doi.org/10.1016/S0169-4332\(02\)01284-9](https://doi.org/10.1016/S0169-4332(02)01284-9).
- Frignani, A., Monticelli, C., Tassinari, M., TrabANELLI, G., Allen, J., Bard, L. R., et al. (2001). *Electrochemical methods: Fundamentals and applications*.
- Fu, K. B., Lin, H., Mo, X. L., Wang, H., Wen, H. W., & Wen, Z. L. (2012). Comparative study on the passivation layers of copper sulphide minerals during bioleaching. *International Journal of Minerals, Metallurgy and Materials*, 19(10), 886–892. <https://doi.org/10.1007/s12613-012-0643-x>.
- Gericke, M., Govender, Y., & Pinches, A. (2010). Tank bioleaching of low-grade chalcopyrite concentrates using redox control. *Hydrometallurgy*, 104(3–4), 414–419. <https://doi.org/10.1016/j.hydromet.2010.02.024>.
- Gerson, A. R., & Bredow, T. (2000). Interpretation of sulphur 2p XPS spectra in sulfide minerals by means of ab initio calculations. *Surface and Interface Analysis*, 29(2), 145–150. [https://doi.org/10.1002/\(SICI\)1096-9918\(200002\)29:2<145::AID-SIA727>3.0.CO;2-N](https://doi.org/10.1002/(SICI)1096-9918(200002)29:2<145::AID-SIA727>3.0.CO;2-N).
- Ghahremaninezhad, A., Dixon, D. G., & Asselin, E. (2013). Electrochemical and XPS analysis of chalcopyrite (CuFeS₂) dissolution in sulfuric acid solution. *Electrochimica Acta*, 87, 97–112. <https://doi.org/10.1016/j.electacta.2012.07.119>.
- Goh, S. W., Buckley, A. N., Lamb, R. N., Rosenberg, R. A., & Moran, D. (2006). The oxidation states of copper and iron in mineral sulfides, and the oxides formed on initial exposure of chalcopyrite and bornite to air. *Geochimica et Cosmochimica Acta*, 70(9), 2210–2228. <https://doi.org/10.1016/j.gca.2006.02.007>.
- Gu, G., Hu, K., Zhang, X., Xiong, X., & Yang, H. (2013). The stepwise dissolution of chalcopyrite bioleached by *Leptospirillum ferriphilum*. *Electrochimica Acta*, 103, 50–57. <https://doi.org/10.1016/j.electacta.2013.04.051>.
- Hackl, R. P., Dreisinger, D. B., Peters, E., & King, J. A. (1995). Passivation of chalcopyrite during oxidative leaching in sulfate media. *Hydrometallurgy*, 39(1–3), 25–48. [https://doi.org/10.1016/0304-386X\(95\)00023-A](https://doi.org/10.1016/0304-386X(95)00023-A).
- Hall, E. H. (1880). On a new action of the magnet on electric currents. *American Journal of Science*, 200–205. <https://doi.org/10.2475/ajs.s3-19.111.200>.
- Hall, S. R., & Stewart, J. M. (1973). The crystal structure refinement of chalcopyrite, CuFeS₂. *Acta Crystallographica Section B Structural Crystallography and Crystal Chemistry*, 579–585. <https://doi.org/10.1107/S0567740873002943>.
- Hamajima, T., Kambara, T., Gondaira, K. I., & Oguchi, T. (1981). Self-consistent electronic structures of magnetic semiconductors by a discrete variational X calculation. III. Chalcopyrite CuFeS₂. *Physical Review B*, 24(6), 3349–3353. <https://doi.org/10.1103/PhysRevB.24.3349>.
- Harmer, S. L., Pratt, A. R., Nesbitt, W. H., & Fleet, M. E. (2004). Sulfur species at chalcopyrite (CuFeS₂) fracture surfaces. *American Mineralogist*, 89(7), 1026–1032. <https://doi.org/10.2138/am-2004-0713>.
- Harmer, S. L., Thomas, J. E., Fornasiero, D., & Gerson, A. R. (2006). The evolution of surface layers formed during chalcopyrite leaching. *Geochimica et Cosmochimica Acta*, 70(17), 4392–4402. <https://doi.org/10.1016/j.gca.2006.06.1555>.

- He, H. (2009). *Studies on the form and transformation of elemental sulfur under the action of typical acidophilic sulfur oxidizing microbes*.
- Hiroyoshi, N., Arai, M., Miki, H., Tsunekawa, M., & Hirajima, T. (2002). A new reaction model for the catalytic effect of silver ions on chalcopyrite leaching in sulfuric acid solutions. *Hydrometallurgy*, 63(3), 257–267. [https://doi.org/10.1016/S0304-386X\(01\)00228-6](https://doi.org/10.1016/S0304-386X(01)00228-6).
- Hiroyoshi, N., Kuroiwa, S., Miki, H., Tsunekawa, M., & Hirajima, T. (2007). Effects of coexisting metal ions on the redox potential dependence of chalcopyrite leaching in sulfuric acid solutions. *Hydrometallurgy*, 87(1–2), 1–10. <https://doi.org/10.1016/j.hydromet.2006.07.006>.
- Hiroyoshi, N., Miki, H., Hirajima, T., & Tsunekawa, M. (2001). Enhancement of chalcopyrite leaching by ferrous ions in acidic ferric sulfate solutions. *Hydrometallurgy*, 60(3), 185–197. [https://doi.org/10.1016/S0304-386X\(00\)00155-9](https://doi.org/10.1016/S0304-386X(00)00155-9).
- Hiskey, J. (1993). Chalcopyrite semiconductor electrochemistry and dissolution. *Extractive Metallurgy of Copper. Nickel and Cobalt*, 1, 949–969.
- Johnson, D. B., & Hallberg, K. B. (2003). The microbiology of acidic mine waters. *Research in Microbiology*, 154.
- Jones, R. (2006). *Electronic structures of the sulfide minerals sphalerite, wurtzite, pyrite, marcasite, and chalcopyrite*.
- Khoshkhoo, M., Dopson, M., Shchukarev, A., & Sandström, Å. (2014). Chalcopyrite leaching and bioleaching: An X-ray photoelectron spectroscopic (XPS) investigation on the nature of hindered dissolution. *Hydrometallurgy*, 149, 220–227. <https://doi.org/10.1016/j.hydromet.2014.08.012>.
- Klauber, C. (2003). Fracture-induced reconstruction of a chalcopyrite (CuFeS₂) surface. *Surface and Interface Analysis*, 35(5), 415–428. <https://doi.org/10.1002/sia.1539>.
- Klauber, C. (2008). A critical review of the surface chemistry of acidic ferric sulphate dissolution of chalcopyrite with regards to hindered dissolution. *International Journal of Mineral Processing*, 86(1–4), 1–17. <https://doi.org/10.1016/j.minpro.2007.09.003>.
- Klauber, C., Parker, A., Van Bronswijk, W., & Watling, H. (2001). Sulphur speciation of leached chalcopyrite surfaces as determined by x-ray photoelectron spectroscopy. *International Journal of Mineral Processing*, 62(1–4), 65–94. [https://doi.org/10.1016/S0301-7516\(00\)00045-4](https://doi.org/10.1016/S0301-7516(00)00045-4).
- Kumar, R. S., Sekar, A., Jaya, N. V., Natarajan, S., & Chichibu, S. (2000). Structural studies of CuAlSe₂ and CuAlS₂ chalcopyrites at high pressures. *Journal of Alloys and Compounds*, 312(1–2), 4–8. [https://doi.org/10.1016/S0925-8388\(00\)00909-9](https://doi.org/10.1016/S0925-8388(00)00909-9).
- Lany, S., Zhao, Y. J., Persson, C., & Zunger, A. (2005). Halogen n-type doping of chalcopyrite semiconductors. *Applied Physics Letters*, 86(4), 1–42109. <https://doi.org/10.1063/1.1854218>.
- Liang, C. L., Xia, J. L., Yang, Y., Nie, Z. Y., Zhao, X. J., Zheng, L., et al. (2011). Characterization of the thermo-reduction process of chalcopyrite at 65 °C by cyclic voltammetry and XANES spectroscopy. *Hydrometallurgy*, 107(1–2), 13–21. <https://doi.org/10.1016/j.hydromet.2011.01.011>.
- Li, C., & Gao, Z. (2018). Tune surface physicochemical property of fluorite particles by regulating the exposure degree of crystal surfaces. *Minerals Engineering*, 128, 123–132. <https://doi.org/10.1016/j.mineng.2018.08.044>.
- Li, A., & Huang, S. (2011). Comparison of the electrochemical mechanism of chalcopyrite dissolution in the absence or presence of *Sulfolobus metallicus* at 70 °C. *Minerals Engineering*, 24(13), 1520–1522. <https://doi.org/10.1016/j.mineng.2011.08.009>.
- Li, J., Kawashima, N., Kaplun, K., Absolon, V. J., & Gerson, A. R. (2010). Chalcopyrite leaching: The rate controlling factors. *Geochimica et Cosmochimica Acta*, 74(10), 2881–2893. <https://doi.org/10.1016/j.gca.2010.02.029>.
- Li, Y., Kawashima, N., Li, J., Chandra, A. P., & Gerson, A. R. (2013). A review of the structure, and fundamental mechanisms and kinetics of the leaching of chalcopyrite. *Advances in Colloid and Interface Science*, 197–198, 1–32. <https://doi.org/10.1016/j.cis.2013.03.004>.
- Linge, H. G. (1976). A study of chalcopyrite dissolution in acidic ferric nitrate by potentiometric titration. *Hydrometallurgy*, 2(1), 51–64. [https://doi.org/10.1016/0304-386X\(76\)90013-X](https://doi.org/10.1016/0304-386X(76)90013-X).
- Liu, H. C., Xia, J. L., & Nie, Z. Y. (2015). Relatedness of Cu and Fe speciation to chalcopyrite bioleaching by *Acidithiobacillus ferrooxidans*. *Hydrometallurgy*, 156, 40–46. <https://doi.org/10.1016/j.hydromet.2015.05.013>.
- Liu, H. C., Xia, J. L., Nie, Z. Y., Wen, W., Yang, Y., Ma, C. Y., et al. (2016). Formation and evolution of secondary minerals during bioleaching of chalcopyrite by thermoacidophilic Archaea *Acidianus manzaensis*. *Transactions of Nonferrous Metals Society of China*, 26(9), 2485–2494. [https://doi.org/10.1016/S1003-6326\(16\)64369-8](https://doi.org/10.1016/S1003-6326(16)64369-8).
- Long, S. J., Jiang, L. S., & Ying, J. C. (2015). Bioleaching of chalcopyrite by thermophilic Archaea. *Advanced Materials Research*, 338–341. <https://doi.org/10.4028/www.scientific.net/amr.1130.338>.
- McMillan, R. S., MacKinnon, D. J., & Dutrizac, J. E. (1982). Anodic dissolution of n-type and p-type chalcopyrite. *Journal of Applied Electrochemistry*, 12(6), 743–757. <https://doi.org/10.1007/BF00617495>.

- Munoz, P. B., Miller, J. D., & Wadsworth, M. E. (1979). Reaction mechanism for the acid ferric sulfate leaching of chalcopyrite. *Metallurgical Transactions B*, 10(2), 149–158. <https://doi.org/10.1007/BF02652458>.
- Mustapha, A.-C., Mehmet, C., & Daniel, S. (1977). *Oxydation ménagée de la chalcopyrite en solution acide : Analyse cinétique des réactions. II. Modèles diffusionnels*. Bulletin de La Société Française de Minéralogie et de Cristallographie. <https://doi.org/10.3406/bulmi.1977.7133>.
- Nava, D., González, I., Leinen, D., & Ramos-Barrado, J. R. (2008). Surface characterization by X-ray photoelectron spectroscopy and cyclic voltammetry of products formed during the potentiostatic reduction of chalcopyrite. *Electrochimica Acta*, 53(14), 4889–4899. <https://doi.org/10.1016/j.electacta.2008.01.088>.
- Ni, Z. Y., Gu, G. H., Yang, H. S., & Qiu, G. Z. (2014). Bioleaching of pyrrhotite by *Sulfobacillus thermosulfidooxidans*. *Journal of Central South University*, 21(7), 2638–2644. <https://doi.org/10.1007/s11771-014-2224-9>.
- Ohgaki, T., Ohashi, N., Sugimura, S., Ryoken, H., Sakaguchi, I., Adachi, Y., et al. (2008). Positive Hall coefficients obtained from contact misplacement on evident n-type ZnO films and crystals. *Journal of Materials Research*, 23(9), 2293–2295. <https://doi.org/10.1557/jmr.2008.0300>.
- Olson, G. J., Brierley, J. A., & Brierley, C. L. (2003). Bioleaching review part B: Progress in bioleaching: Applications of microbial processes by the minerals industries. *Applied Microbiology and Biotechnology*, 63(3), 249–257. <https://doi.org/10.1007/s00253-003-1404-6>.
- Parker, A., Klauber, C., Kougiannos, A., Watling, H. R., & Van Bronswijk, W. (2003). An X-ray photoelectron spectroscopy study of the mechanism of oxidative dissolution of chalcopyrite. *Hydrometallurgy*, 71(1–2), 265–276. [https://doi.org/10.1016/S0304-386X\(03\)00165-8](https://doi.org/10.1016/S0304-386X(03)00165-8).
- Parker, A. J., Paul, R. L., & Power, G. P. (1981). Electrochemistry of the oxidative leaching of copper from chalcopyrite. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, 305–316. [https://doi.org/10.1016/S0022-0728\(81\)80549-9](https://doi.org/10.1016/S0022-0728(81)80549-9).
- Petersen, J., & Dixon, D. G. (2006). Competitive bioleaching of pyrite and chalcopyrite. *Hydrometallurgy*, 83(1–4), 40–49. <https://doi.org/10.1016/j.hydromet.2006.03.036>.
- Pradhan, N., Nathsarma, K. C., Srinivasa Rao, K., Sukla, L. B., & Mishra, B. K. (2008). Heap bioleaching of chalcopyrite: A review. *Minerals Engineering*, 21(5), 355–365. <https://doi.org/10.1016/j.mineng.2007.10.018>.
- Qin, W., Yang, C., Lai, S., Wang, J., Liu, K., & Zhang, B. (2013). Bioleaching of chalcopyrite by moderately thermophilic microorganisms. *Bioresource Technology*, 129, 200–208. <https://doi.org/10.1016/j.biortech.2012.11.050>.
- Qin, W., Zhen, S., Yan, Z., Campbell, M., Wang, J., Liu, K., et al. (2009). Heap bioleaching of a low-grade nickel-bearing sulfide ore containing high levels of magnesium as olivine, chlorite and antigorite. *Hydrometallurgy*, 98(1–2), 58–65. <https://doi.org/10.1016/j.hydromet.2009.03.017>.
- Rais, A., Gismelseed, A. M., & Al-Rawas, A. D. (2000). Magnetic properties of natural chalcopyrite at low temperature. *Materials Letters*, 46(6), 349–353. [https://doi.org/10.1016/S0167-577X\(00\)00199-3](https://doi.org/10.1016/S0167-577X(00)00199-3).
- Rashkeev, S. N., & Lambrecht, W. R. (2001). Second-harmonic generation of I-III-VI 2 chalcopyrite semiconductors: Effects of chemical substitutions. *Physical Review B*, 63.
- Rohwerder, T., Gehrke, T., Kinzler, K., & Sand, W. (2003). *Bioleaching review part A: Applied microbiology and biotechnology*. <https://doi.org/10.1007/s00253-003-1448-7>.
- Roy, M. S. (1978). Study of semiconductors using electrochemical techniques. *Journal of Vacuum Science and Technology*, 1417–1421. <https://doi.org/10.1116/1.569799>.
- Sandström, A., Shchukarev, A., & Paul, J. (2005). XPS characterisation of chalcopyrite chemically and bio-leached at high and low redox potential. *Minerals Engineering*, 18(5), 505–515. <https://doi.org/10.1016/j.mineng.2004.08.004>.
- Sasaki, K., Nakamuta, Y., Hirajima, T., & Tuovinen, O. H. (2009). Raman characterization of secondary minerals formed during chalcopyrite leaching with *Acidithiobacillus ferrooxidans*. *Hydrometallurgy*, 95(1–2), 153–158. <https://doi.org/10.1016/j.hydromet.2008.05.009>.
- Schippers, A. (2007). Microorganisms involved in bioleaching and nucleic acid-based molecular methods for their identification and quantification. In *Microbial processing of metal sulfides* (pp. 3–33). Springer Netherlands. https://doi.org/10.1007/1-4020-5589-7_1..
- Shirley, D. A. (1972). High-resolution x-ray photoemission spectrum of the valence bands of gold. *Physical Review B*, 5(12), 4709–4714. <https://doi.org/10.1103/PhysRevB.5.4709>.
- Sieberttritt, S., Igalson, M., Persson, C., & Lany, S. (2010). The electronic structure of chalcopyrites - bands, point defects and grain boundaries. *Progress in Photovoltaics: Research and Applications*, 18(6), 390–410. <https://doi.org/10.1002/pip.936>.

- Smart, R. S. C., Skinner, W. M., & Gerson, A. R. (1999). XPS of sulphide mineral surfaces: Metal-deficient, polysulphides, defects and elemental sulphur. *Surface and Interface Analysis*, 28(1), 101–105. [https://doi.org/10.1002/\(SICI\)1096-9918\(199908\)28:1<101::AID-SIA627>3.0.CO;2-0](https://doi.org/10.1002/(SICI)1096-9918(199908)28:1<101::AID-SIA627>3.0.CO;2-0).
- Su, D. S., Neumann, W., & Giersig, M. (2000). Structure modifications in chalcopyrite semiconductors. *Thin Solid Films*, 361, 218–222. [https://doi.org/10.1016/S0040-6090\(99\)00837-8](https://doi.org/10.1016/S0040-6090(99)00837-8).
- Teranishi, T., & Sato, K. (1975). Optical, electrical and magnetic properties of chalcopyrite. *CuFeS₂*. *Le Journal de Physique Colloques*, 36, 3–149.
- Third, K. A., Cord-Ruwisch, R., & Watling, H. R. (2000). Role of iron-oxidizing bacteria in stimulation or inhibition of chalcopyrite bioleaching. *Hydrometallurgy*, 57(3), 225–233. [https://doi.org/10.1016/S0304-386X\(00\)00115-8](https://doi.org/10.1016/S0304-386X(00)00115-8).
- Third, K. A., Cord-Ruwisch, R., & Watling, H. R. (2002). Control of the redox potential by oxygen limitation improves bacterial leaching of chalcopyrite. *Biotechnology and Bioengineering*, 78(4), 433–441. <https://doi.org/10.1002/bit.10184>.
- Todd, E. C., Sherman, D. M., & Purton, J. A. (2003). Surface oxidation of chalcopyrite (CuFeS₂) under ambient atmospheric and aqueous (pH 2–10) conditions: Cu, Fe L- and O K-edge X-ray spectroscopy. *Geochimica et Cosmochimica Acta*, 67(12), 2137–2146. [https://doi.org/10.1016/S0016-7037\(02\)01371-6](https://doi.org/10.1016/S0016-7037(02)01371-6).
- Tributsch, H., & Bennett, J. C. (1981). Semiconductor-electrochemical aspects of bacterial leaching - 1. Oxidation of metal sulphides with large energy gaps. *Journal of Chemical Technology and Biotechnology*, 31(9), 565–577. <https://doi.org/10.1002/jctb.503310176>.
- Velásquez, P., Leinen, D., Pascual, J., Ramos-Barrado, J. R., Grez, P., Gómez, H., et al. (2005). A chemical, morphological, and electrochemical (XPS, SEM/EDX, CV, and EIS) analysis of electrochemically modified electrode surfaces of natural chalcopyrite (CuFeS₂) and pyrite (FeS₂) in alkaline solutions. *Journal of Physical Chemistry B*, 109(11), 4977–4988. <https://doi.org/10.1021/jp048273u>.
- Vilcáez, J., Suto, K., & Inoue, C. (2008). Bioleaching of chalcopyrite with thermophiles: Temperature-pH-ORP dependence. *International Journal of Mineral Processing*, 88(1–2), 37–44. <https://doi.org/10.1016/j.minpro.2008.06.002>.
- Vilcáez, J., Yamada, R., & Inoue, C. (2009). Effect of pH reduction and ferric ion addition on the leaching of chalcopyrite at thermophilic temperatures. *Hydrometallurgy*, 96(1–2), 62–71. <https://doi.org/10.1016/j.hydromet.2008.08.003>.
- Von Oertzen, G. U., Harmer, S. L., & Skinner, W. M. (2006). XPS and ab initio calculation of surface states of sulfide minerals: Pyrite, chalcopyrite and molybdenite. *Molecular Simulation*, 32(15), 1207–1212. <https://doi.org/10.1080/08927020601081616>.
- Warren, G. W., Sohn, H. J., Wadsworth, M. E., & Wang, T. G. (1985). The effect of electrolyte composition on the cathodic reduction of CuFeS₂. *Hydrometallurgy*, 14(2), 133–149. [https://doi.org/10.1016/0304-386X\(85\)90030-1](https://doi.org/10.1016/0304-386X(85)90030-1).
- Watling, H. R. (2006). The bioleaching of sulphide minerals with emphasis on copper sulphides - a review. *Hydrometallurgy*, 84(1–2), 81–108. <https://doi.org/10.1016/j.hydromet.2006.05.001>.
- Woolley, J. C., Lamarche, A. M., Lamarche, G., Quintero, M., Swainson, I. P., & Holden, T. M. (1996). Low temperature magnetic behaviour of CuFeS₂ from neutron diffraction data. *Journal of Magnetism and Magnetic Materials*, 162(2–3), 347–354. [https://doi.org/10.1016/S0304-8853\(96\)00252-1](https://doi.org/10.1016/S0304-8853(96)00252-1).
- Xue, D., Betzler, K., & Hesse, H. (2000). Dielectric properties of I-III-VI 2-type chalcopyrite semiconductors. *Physical Review B*, 62.
- Yang, C., Qin, W., Lai, S., Wang, J., Zhang, Y., Jiao, F., et al. (2011). Bioleaching of a low grade nickel-copper-cobalt sulfide ore. *Hydrometallurgy*, 106(1–2), 32–37. <https://doi.org/10.1016/j.hydromet.2010.11.013>.
- Yang, Y., Weihua, L., & Miao, C. (2013). A copper and iron K-edge XANES study on chalcopyrite leached by mesophiles and moderate thermophiles. *Minerals Engineering*, 31–35. <https://doi.org/10.1016/j.mineng.2013.01.010>.
- Yang, Y., Harmer, S., & Chen, M. (2015). Synchrotron-based XPS and NEXAFS study of surface chemical species during electrochemical oxidation of chalcopyrite. *Hydrometallurgy*, 156, 89–98. <https://doi.org/10.1016/j.hydromet.2015.05.011>.
- Yin, Q., Kelsall, G. H., Vaughan, D. J., & England, K. E. R. (1995). Atmospheric and electrochemical oxidation of the surface of chalcopyrite (CuFeS₂). *Geochimica et Cosmochimica Acta*, 59(6), 1091–1100. [https://doi.org/10.1016/0016-7037\(95\)00026-V](https://doi.org/10.1016/0016-7037(95)00026-V).
- Yin, M., Wu, C. K., Lou, Y., Burda, C., Koberstein, J. T., Zhu, Y., et al. (2005). Copper oxide nanocrystals. *Journal of the American Chemical Society*, 127(26), 9506–9511. <https://doi.org/10.1021/ja050006u>.
- Zeng, W. (2011). *The formation mechanism of passivation layer and its elimination way during bioleaching of chalcopyrite*.
- Zhang. (2011). *Study on chemical transformation on the surface of chalcopyrite during the bioleaching process*.

- Zhang, L., Wu, J., Wang, Y., Wan, L., Mao, F., Zhang, W., et al. (2014). Influence of bioaugmentation with *Ferroplasma thermophilum* on chalcopyrite bioleaching and microbial community structure. *Hydrometallurgy*, 146, 15–23. <https://doi.org/10.1016/j.hydromet.2014.02.013>.
- Zhang, Y., Zhao, G., Lv, X., Tian, Y., Yang, L., Zou, G., et al. (2019). Exploration and size engineering from natural chalcopyrite to high-performance electrode materials for lithium-ion batteries. *ACS Applied Materials and Interfaces*. <https://doi.org/10.1021/acsami.8b22094>.
- Zhao, H., Wang, J., Hu, M., Qin, W., Zhang, Y., & Qiu, G. (2013). Synergistic bioleaching of chalcopyrite and bornite in the presence of *Acidithiobacillus ferrooxidans*. *Bioresource Technology*, 149, 71–76. <https://doi.org/10.1016/j.biortech.2013.09.035>.
- Zhao, H., Wang, J., Qin, W., Hu, M., Zhu, S., & Qiu, G. (2015). Electrochemical dissolution process of chalcopyrite in the presence of mesophilic microorganisms. *Minerals Engineering*, 71, 159–169. <https://doi.org/10.1016/j.mineng.2014.10.025>.
- Zhao, H., Zhang, Y., Zhang, X., Qian, L., Sun, M., Yang, Y., et al. (2019). The dissolution and passivation mechanism of chalcopyrite in bioleaching: An overview. *Minerals Engineering*, 136, 140–154. <https://doi.org/10.1016/j.mineng.2019.03.014>.
- Zhou, H. B., Zeng, W. M., Yang, Z. F., Xie, Y. J., & Qiu, G. Z. (2009). Bioleaching of chalcopyrite concentrate by a moderately thermophilic culture in a stirred tank reactor. *Bioresource Technology*, 100(2), 515–520. <https://doi.org/10.1016/j.biortech.2008.06.033>.
- Zhu, W., Xia, J.L., Yang, Y., Nie, Z.y., Zheng, L., Ma, C.y., et al. (2011). Sulfur oxidation activities of pure and mixed thermophiles and sulfur speciation in bioleaching of chalcopyrite. *Bioresource Technology*, 102(4), 3877–3882. <https://doi.org/10.1016/j.biortech.2010.11.090>.

Role of gangue minerals in chalcopyrite bioleaching

Chalcopyrite is commonly associated with the other minerals, especially sulfides. The purity or mineralogical composition of chalcopyrite ore should be characterized before bioleaching because associated minerals or impurities can affect the dissolution mechanism of chalcopyrite in bioleaching. Gangue minerals, including silicates and sulfide minerals, are commonly associated with chalcopyrite in raw ores. The solubilization of silicates consumes acid and releases elements such as Si, Al, Mg, F^- , and Cl^- , affecting chalcopyrite bioleaching (Konishi et al., 1999). In addition, a galvanic effect often exists between gangue sulfide minerals and chalcopyrite in bioleaching and has been widely reported in chalcopyrite leaching. For example, pyrite with a higher rest potential can act as the cathode and promote the oxidation of chalcopyrite (anode). Some studies found that the auxo-action of pyrite for chalcopyrite leaching was significant in ferric sulfate media, and a process known as Galvanox was developed (Dixon et al., 2008; Nazari et al., 2011, 2012a, 2012b). Except for the galvanic effect, gangue sulfide minerals can influence the biosolubilization process of chalcopyrite by releasing metal ions and altering aqueous chemistry conditions. Hence, this work studied the role of gangue minerals in chalcopyrite bioleaching.

5.1 Role of marmatite in chalcopyrite bioleaching

5.1.1 Bioleaching behavior

Pure chalcopyrite and marmatite samples were obtained from the geological museum of Hunan Province of China and Dachang Mine of Guangxi Province of China, respectively. X-ray diffraction indicated that they were both of high purity. The silver-bearing zinc leaching residue (ZLR) that was chosen was from a smelting plant in Chenzhou, Hunan Province, China. Ore samples were all ground and sieved to less than 0.074 mm before they were used for leaching experiments and analysis. All chemicals used in this work were of analytical grade.

Moderately thermophilic microorganisms of *Acidithiobacillus caldus* (CCTC AB 206240) and *Leptospirillum ferriphilum* (CCTC AB 206239) were initially obtained from the Key Lab of

Bio-hydrometallurgy of Ministry of Education, Central South University, Changsha, China. Bioleaching experiments of mixed ores with different mass ratios of chalcopyrite and marmatite were carried out at 170 rpm, 45°C, pH 1.70. After solid–liquid separation, the further recovery of Cu from residues was conducted by catalytic bioleaching in the presence of silver-bearing ZLR. Metal ions concentrations were analyzed through inductively coupled plasma-atomic emission spectrometer (America Baird Co. PS-6). The pH value was monitored with a pH meter (PHSJ-4A) and the redox potential was detected by an oxidation-reduction potential (ORP) meter (BPH-221).

Fig. 5.1 shows the leaching results of Cu–Zn mixed ores consisting of different ratios of chalcopyrite and marmatite by sterile culture medium, *L. ferriphilum*, mixed culture of *L. ferriphilum* and *A. caldus*, and mixed culture of *L. ferriphilum* and *A. caldus* with the addition of Fe^{3+} , respectively. The copper extraction of chalcopyrite significantly decreased with the addition of marmatite, but the zinc extraction of marmatite remarkably increased owing to the presence of chalcopyrite, and marmatite dissolved remarkably faster than chalcopyrite. Hence, stepwise bioleaching was feasible for Cu–Zn mixed ores of chalcopyrite and marmatite, especially for mixed ores consisting of a high mass ratio of chalcopyrite and a low mass ratio of marmatite. In addition, the stepwise dissolution trend became more obvious according to the order of sterile leaching: bioleaching by *L. ferriphilum*, bioleaching by a mixed culture of *L. ferriphilum* and *A. caldus*, and bioleaching by a mixed culture of *L. ferriphilum* and *A. caldus* with added Fe^{3+} . The bioleaching system of a mixed culture with added Fe^{3+} was the most effective for the stepwise bioleaching of Cu–Zn mixed ores, but the Fe^{3+} concentration should be appropriate to promote marmatite dissolution effectively.

Fig. 5.2 shows variations in the redox potential during the leaching of Cu–Zn mixed ores consisting of different ratios of chalcopyrite and marmatite by sterile culture medium, *L. ferriphilum*, a mixed culture of *L. ferriphilum* and *A. caldus*, and a mixed culture of *L. ferriphilum* and *A. caldus* with added Fe^{3+} , respectively. The redox potentials steadily increased during the whole sterile leaching process and were low (less than 380 mV versus Ag/AgCl). However, during bioleaching, the redox potentials sharply increased at the initial stage and reached a plateau at the final stage. A high redox potential should be maintained to make marmatite preferentially dissolve and accomplish stepwise bioleaching. This is why the bioleaching system of a mixed culture with added Fe^{3+} was the most effective for the stepwise bioleaching of Cu–Zn mixed ores.

5.1.2 Intermediate species analysis

Leaching results indicated that stepwise bioleaching was more effective for mixed ores with a high chalcopyrite–marmatite ratio. Hence, the transformation and distribution of intermediate species on the surface of Cu–Zn mixed samples (chalcopyrite–marmatite ratio of 5:1) during leaching were investigated by X-ray photoelectron spectroscopic (XPS) analysis. XPS peaks were fitted by Thermo Advantage 5.52 software. The XPS spectra of mixed samples leached in four systems (sterile culture medium, *L. ferriphilum*, a mixed culture of *L. ferriphilum* and *A. caldus*, and a mixed culture of *L. ferriphilum* and *A. caldus* with Fe^{3+}) for different days were analyzed. The Cu $2p_{3/2}$ peak (Fig. 5.3) was commonly used as the characteristic peak to judging Cu valence states and species. The binding energy of about 933.0–933.8 eV with the existence of a shake-up peak (about 939.0–944.0 eV) are the two

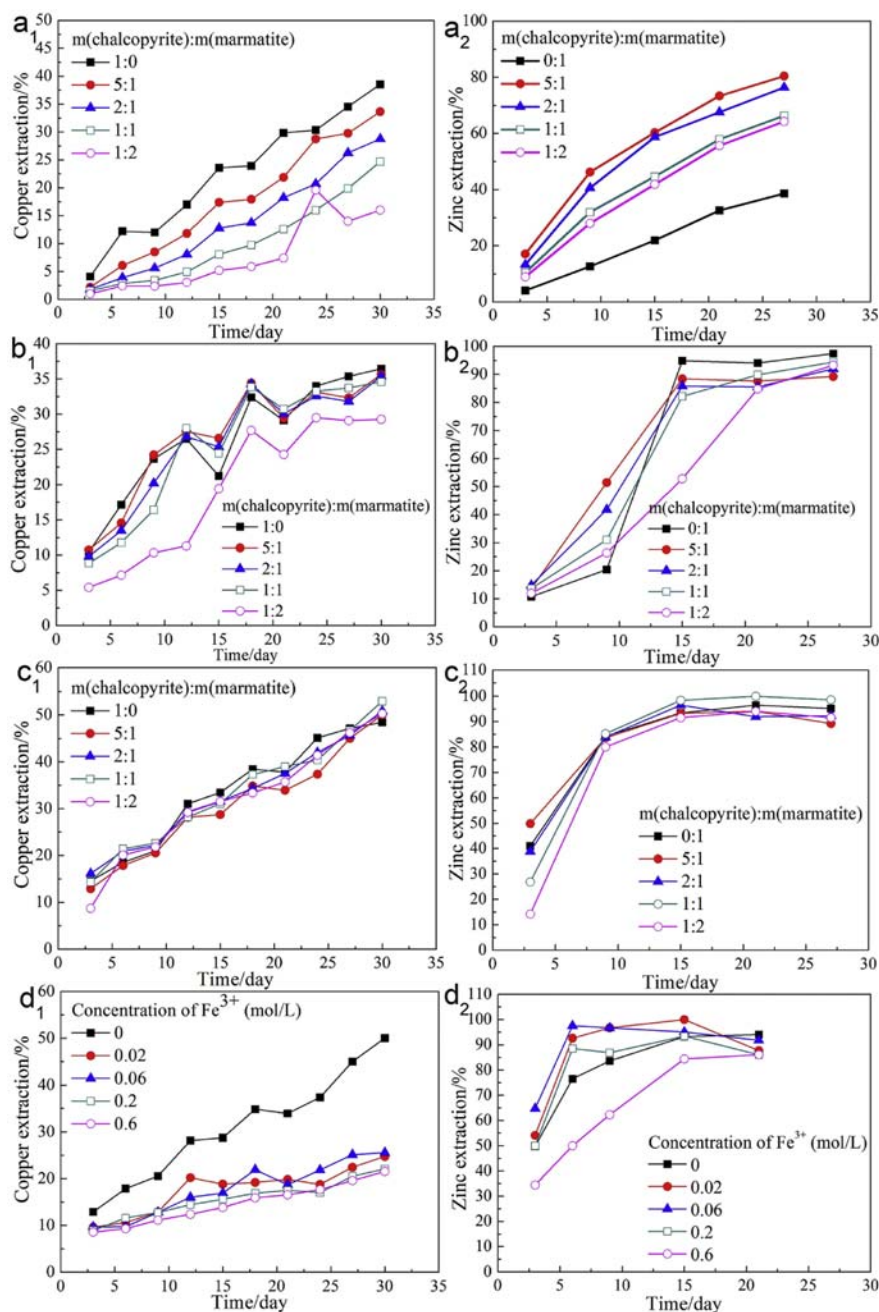


FIGURE 5.1 Copper and zinc extractions during leaching of mixed ores consisting of different ratios of chalcopyrite and marmatite: (a) Sterile leaching; (b) *L. ferriphilum*; (c) Mixed culture of *L. ferriphilum* and *A. caldus*; (d) Mixed culture of *L. ferriphilum* and *A. caldus* with Fe^{3+} addition. From Zhao, H., Gan, X., Wang, J., Tao, L., Qin, W., & Qiu, G. (2017). Stepwise bioleaching of Cu-Zn mixed ores with comprehensive utilization of silver-bearing solid waste through a new technique process. *Hydrometallurgy*, 171, 374–386. <https://doi.org/10.1016/j.hydromet.2017.06.002>.

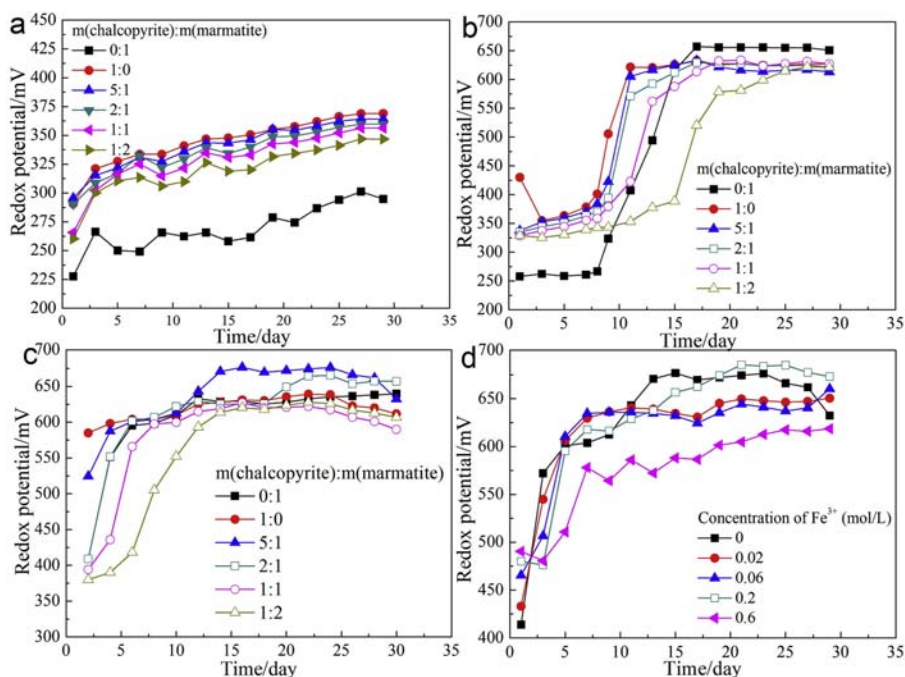


FIGURE 5.2 Variations in redox potential during leaching of mixed ores consisting of different ratios of chalcopyrite and marmatite. (A) Sterile leaching; (B) *Leptospirillum ferriphilum*; (C) Mixed culture of *L. ferriphilum* and *Acidithiobacillus caldus*; (D) Mixed culture of *L. ferriphilum* and *A. caldus* with Fe^{3+} addition. From Zhao, H., Gan, X., Wang, J., Tao, L., Qin, W., & Qiu, G. (2017). Stepwise bioleaching of Cu-Zn mixed ores with comprehensive utilization of silver-bearing solid waste through a new technique process. *Hydrometallurgy*, 171, 374–386. <https://doi.org/10.1016/j.hydromet.2017.06.002>.

main XPS characteristics of cupric species, and a lower binding energy value (about 931.8–933.1 eV) of the Cu $2p_{3/2}$ peak with the absence of a shake-up peak are the main features of the cuprous species of Cu_2O (932.4 eV), CuS (932.0–932.4 eV), and Cu_2S (932.9 eV) (Ghahremaninezhad et al., 2013; Li et al., 2013; Nakai et al., 1978). Cu $2p_{3/2}$ peaks of all samples were centered at the range of 932.0–932.4 eV, which indicates that cuprous species consisting of CuS are the most plausible copper-bearing intermediate species in these four leaching systems.

Zn $2p$ peaks of leached samples (chalcopyrite to marmatite ratio of 5:1) presented in Fig. 5.4 show that the Zn $2p_{3/2}$ peaks were centered at about 1021.5–1022.5 eV, which was in agreement with the binding energy of zinc sulfide or zinc hydroxide species (Boulton et al., 2003; Harmer et al., 2007, 2008). Hence, zinc sulfide and zinc hydroxide species were the most plausible zinc-bearing intermediate species.

Sulfur species were considered to be the main passivating species in the hydrometallurgical process of chalcopyrite, even though the specific passivating species and detailed compositions of passivating layer are still debated. For S $2p$ peak fitting, the S $2p$ peaks appeared as doublets with S $2p_{3/2}$ and S $2p_{1/2}$ owing to spin-orbit splitting and were fitted with a 2:1 peak area ratio and 1.2 eV splitting for S $2p_{3/2}$ and S $2p_{1/2}$. The S $2p$ spectra were fitted by the Gaussian–Lorentzian line function, and the Shirley method was applied to obtain the

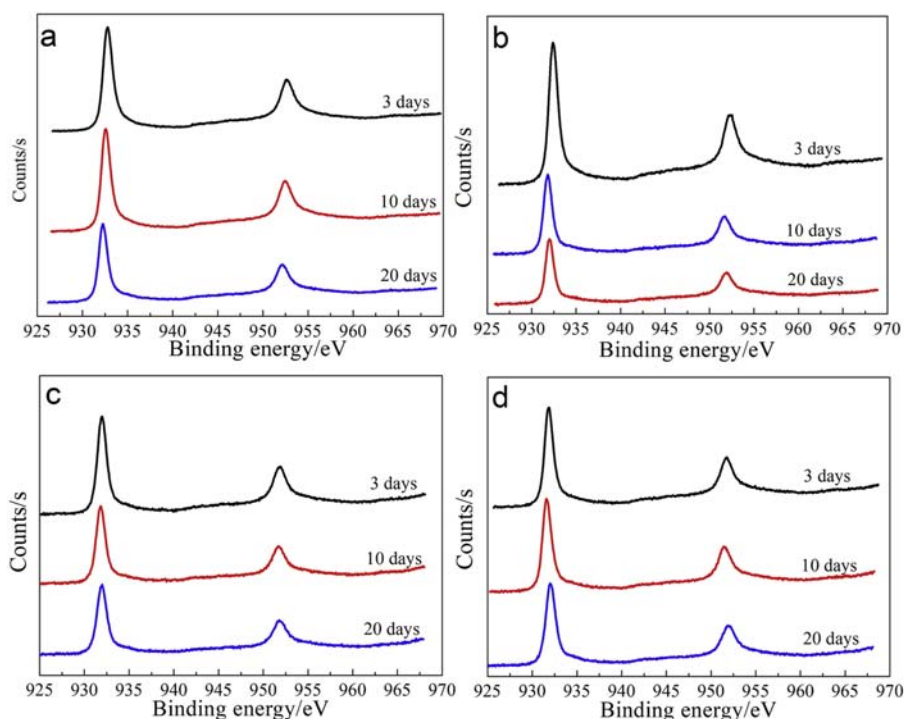


FIGURE 5.3 X-ray photoelectron spectroscopic spectra of Cu 2p peaks of Cu-Zn mixed samples (chalcopyrite/marmatite is 5:1) leached for 3, 10, and 20 days. (A) Sterile leaching; (B) *Leptospirillum ferriphilum*; (C) Mixed culture of *L. ferriphilum* and *Acidithiobacillus caldus*; (D) Mixed culture of *L. ferriphilum* and *A. caldus* with Fe^{3+} addition. From Zhao, H., Gan, X., Wang, J., Tao, L., Qin, W., & Qiu, G. (2017). Stepwise bioleaching of Cu-Zn mixed ores with comprehensive utilization of silver-bearing solid waste through a new technique process. *Hydrometallurgy*, 171, 374–386. <https://doi.org/10.1016/j.hydromet.2017.06.002>.

background of spectra (Shirley, 1972). Fig. 5.5 shows the S 2p peaks of Cu–Zn mixed samples (chalcopyrite to marmatite ratio of 5:1) leached for 3, 10, and 20 days. The corresponding binding energy and full width at half-maximum (FWHM) values are presented in Table 5.1. The S 2p_{3/2} peaks were fitted and the corresponding binding energy and FWHM values were compared with references (Buckley & Woods, 1984; Ghahremaninezhad et al., 2013; Harmer et al., 2006; Klauber et al., 2001). Disulfide (S_2^{2-}), monosulfide (S_2^{-}), polysulfide (S_n^{2-}), and elemental sulfur (S^0) were the main sulfur species during sterile leaching. Besides S_2^{2-} , S_2^{-} , S^0 , and S_n^{2-} , sulfate (SO_4^{2-}) formed at a later stage of bioleaching by *L. ferriphilum*. S_2^{2-} , S_2^{-} , S^0 , S_n^{2-} , and SO_4^{2-} were the main sulfur species during the whole bioleaching process in the presence of a mixed culture.

The distribution of sulfur species on the surface of Cu–Zn mixed samples (chalcopyrite to marmatite ratio of 5:1) leached for 3, 10, and 20 days is shown in Fig. 5.6. In sterile leaching and at the initial stage of bioleaching by *L. ferriphilum*, the percentage of different sulfur species decreased according to the order of S_2^{2-} , S_2^{-} , S_n^{2-} , and S^0 . In bioleaching by mixed culture and at the later stage of bioleaching by *L. ferriphilum*, S_2^{2-} and SO_4^{2-} were the dominant sulfur species, followed by S_2^{-} , S_n^{2-} , and S^0 . Hence, XPS analysis proved that the types of

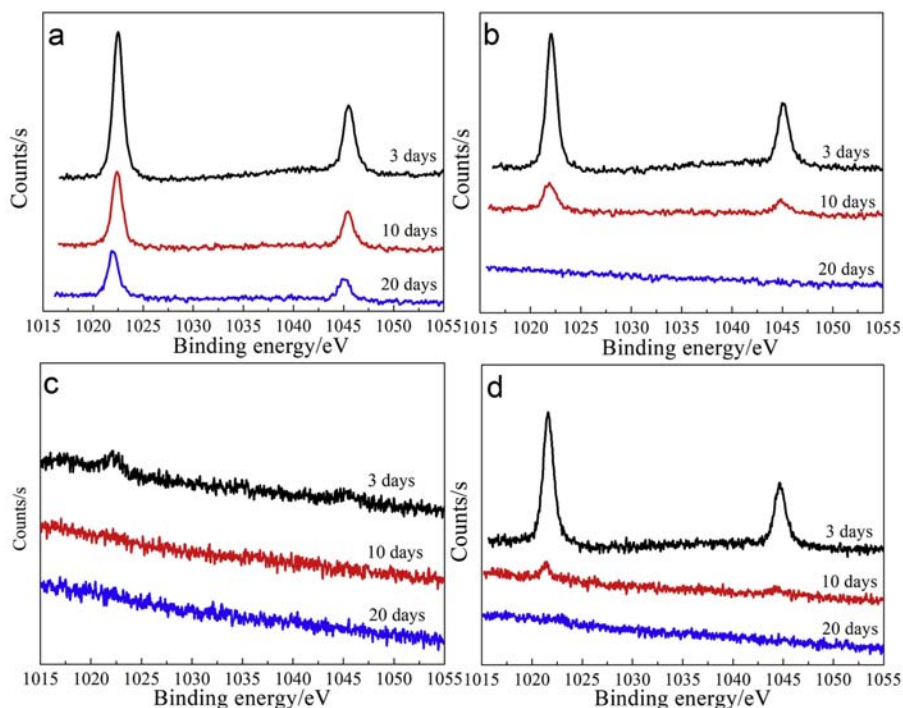


FIGURE 5.4 X-ray photoelectron spectroscopic spectra of Zn 2p peaks of Cu-Zn mixed samples (chalcopyrite/marmatite is 5:1) leached by for 3, 10, and 20 days. (A) Sterile leaching; (B) *Leptospirillum ferriphilum*; (C) Mixed culture of *L. ferriphilum* and *Acidithiobacillus caldus*; (D) Mixed culture of *L. ferriphilum* and *A. caldus* with Fe^{3+} addition. From Zhao, H., Gan, X., Wang, J., Tao, L., Qin, W., & Qiu, G. (2017). Stepwise bioleaching of Cu-Zn mixed ores with comprehensive utilization of silver-bearing solid waste through a new technique process. *Hydrometallurgy*, 171, 374–386. <https://doi.org/10.1016/j.hydromet.2017.06.002>.

intermediate species were similar in the bioleaching of Cu–Zn mixed samples of chalcopyrite and marmatite, indicating that the dissolution processes of Cu–Zn mixed samples in different bioleaching systems were similar.

S^{2-} , S_2^{2-} , S_n^{2-} , S^0 , and SO_4^{2-} were common sulfur-containing species on a mineral surface in chalcopyrite bioleaching. The main sulfur-containing species of untreated chalcopyrite was S^{2-} , which could be partly transformed to S_2^{2-} owing to surface reconstruction (De Oliveira et al., 2012). The formed S_2^{2-} could be transformed to S_n^{2-} under an oxidizing atmosphere, and S_n^{2-} could be further oxidized to S^0 and SO_4^{2-} sequentially when an oxidizing atmosphere was relatively stronger (Ghahremaninezhad et al., 2010; Klauber, 2008). In this work, the total percentage of S_n^{2-} , S^0 , and SO_4^{2-} increased in accordance with the order of sterile leaching, *L. ferriphilum*, mixed culture, and a mixed culture (0.06 mol/L Fe^{3+}) system, because the oxidizing atmosphere increased in accordance with that order. The redox potential can be used to represent the oxidizing atmosphere in a bioleaching system. It has been reported that S_n^{2-} , S^0 , and SO_4^{2-} were possible passivating species in chalcopyrite bioleaching. A high redox potential promoted the formation of passivating species and easily caused the passivation of chalcopyrite (Khoshkhoo et al., 2014; Sandström et al., 2005; Yang et al., 2015). That is why a high redox potential

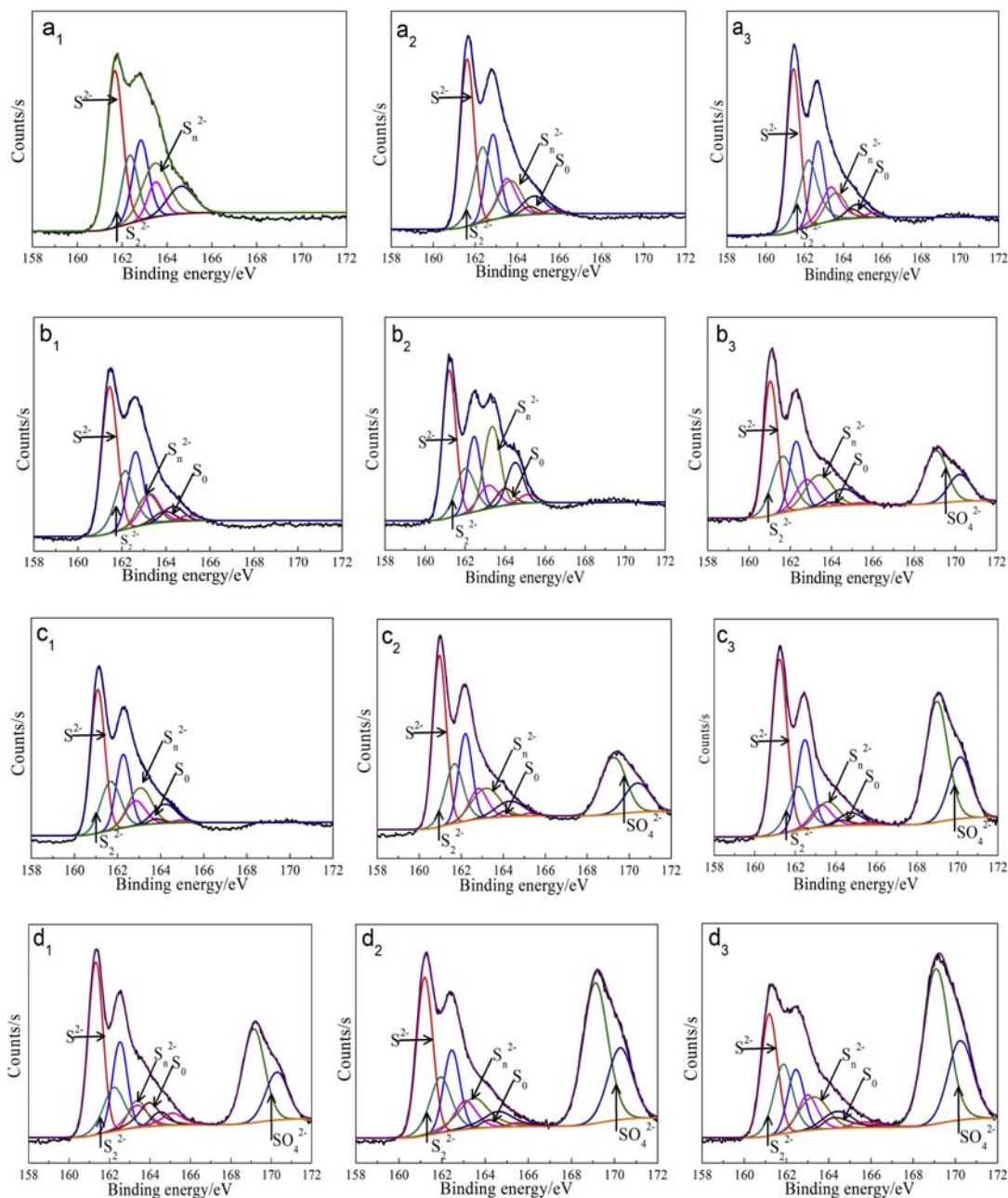


FIGURE 5.5 X-ray photoelectron spectroscopic spectra of fitted S 2p peaks of Cu-Zn mixed samples (chalcopyrite/marmatite is 5:1) leached for 3, 10, and 20 days. (A) Sterile leaching; (B) *Leptospirillum ferriphilum*; (C) Mixed culture of *L. ferriphilum* and *Acidithiobacillus caldus*; (D) Mixed culture of *L. ferriphilum* and *A. caldus* with Fe^{3+} addition. From Zhao, H., Gan, X., Wang, J., Tao, L., Qin, W., & Qiu, G. (2017). Stepwise bioleaching of Cu-Zn mixed ores with comprehensive utilization of silver-bearing solid waste through a new technique process. *Hydrometallurgy*, 171, 374–386. <https://doi.org/10.1016/j.hydromet.2017.06.002>.

TABLE 5.1 Binding energy (B.E.) and full width at half-maximum FWHM value for X-ray photoelectron spectra of S 2p_{3/2} peaks of Cu–Zn mixed samples (chalcopyrite to marmatite ratio of 5:1) leached for 3, 10, and 20 days.

Conditions, d	Peak 1			Peak 2		Peak 3		Peak 4		Peak 5		
	B.E./eV	FWHM/		B.E./eV	FWHM/eV	B.E./eV	FWHM/		B.E./eV	FWHM/eV	B.E./eV	FWHM/eV
		eV					eV					
a	3	161.68	0.84	162.35	0.83	163.84	1.38	—	—	—	—	
	10	161.61	0.78	162.34	1.01	163.62	1.28	164.59	0.87	—	—	
	20	161.43	0.80	162.20	1.12	163.56	1.23	164.41	0.93	—	—	
b	3	161.44	0.83	162.15	0.99	163.15	1.10	163.92	1.10	—	—	
	10	161.21	0.78	162.01	1.14	163.33	0.96	163.97	1.00	—	—	
	20	161.03	0.80	161.63	1.21	163.37	1.56	164.07	0.86	169.06	1.27	
c	3	161.31	0.86	162.22	1.23	163.32	1.03	163.96	1.23	169.12	1.29	
	10	161.20	0.89	161.93	1.16	163.44	1.65	164.20	0.92	169.11	1.36	
	20	161.18	0.92	161.84	1.05	163.24	1.62	164.21	1.42	169.06	1.41	
d	3	161.09	0.77	161.67	1.09	163.02	1.27	163.78	0.77	—	—	
	10	160.96	0.76	161.66	1.01	163.14	1.57	164.04	0.58	169.22	1.41	
	20	161.21	0.89	162.14	1.15	163.60	1.38	164.24	0.97	168.98	1.29	

(a) Sterile leaching; (b) *Leptospirillum ferriphilum*; (c) Mixed culture of *L. ferriphilum* and *Acidithiobacillus caldus*; (d) Mixed culture of *L. ferriphilum* and *A. caldus* with ferric ions added.

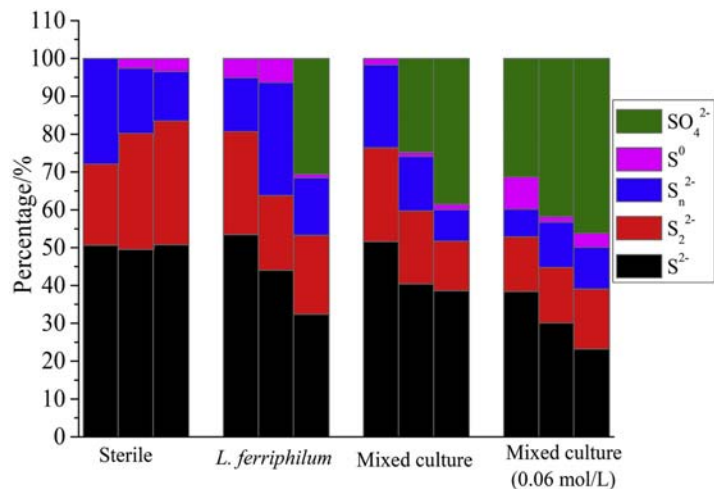


FIGURE 5.6 Distributions of sulfur species on the surface of Cu–Zn mixed samples (chalcopyrite to marmatite ratio of 5:1) leached for 3, 10, and 20 days. From Zhao, H., Gan, X., Wang, J., Tao, L., Qin, W., & Qiu, G. (2017). Stepwise bioleaching of Cu–Zn mixed ores with comprehensive utilization of silver-bearing solid waste through a new technique process. *Hydrometallurgy*, 171, 374–386. <https://doi.org/10.1016/j.hydromet.2017.06.002>.

should be maintained to make marmatite preferentially dissolve and accomplish stepwise bioleaching, and why the bioleaching system of a mixed culture with Fe^{3+} was most effective for the stepwise bioleaching of Cu–Zn mixed ores.

5.1.3 Electrochemistry analysis

A carbon paste electroactive electrode consisting of 0.7 g of minerals (–0.038 mm), 0.2 g of graphite, and 0.1 g of solid paraffin was used in electrochemistry analysis. The electrodes were all polished by silicon carbide paper to obtain a smooth surface before each electrochemical measurement. Electrochemistry tests were conducted by a conventional three-electrode system (Princeton Model 283 Potentiostat, EG&G of Princeton Applied Research). All potential values mentioned refer to the Ag/AgCl electrode (versus Ag/AgCl) in this work.

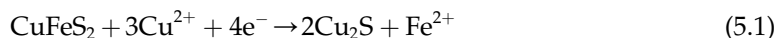


Fig. 5.7(a₁–a₂) shows the cyclic voltammety spectra of Cu–Zn mixed samples with different ratios of chalcopyrite and marmatite. Two scan routes carried out: the positive scan route started scanning toward the positive direction and the negative scan route started toward the negative direction. The corresponding reactions of anodic-cathodic peaks (a–h) of chalcopyrite are shown as follows from Eqs. (5.1–5.8) (Liang, Xia, Yang, & Nie, 2011; Gu, Hu, Zhang, Xiong, & Yang, 2013; Arce & González, 2002). Anodic peak h can also represent

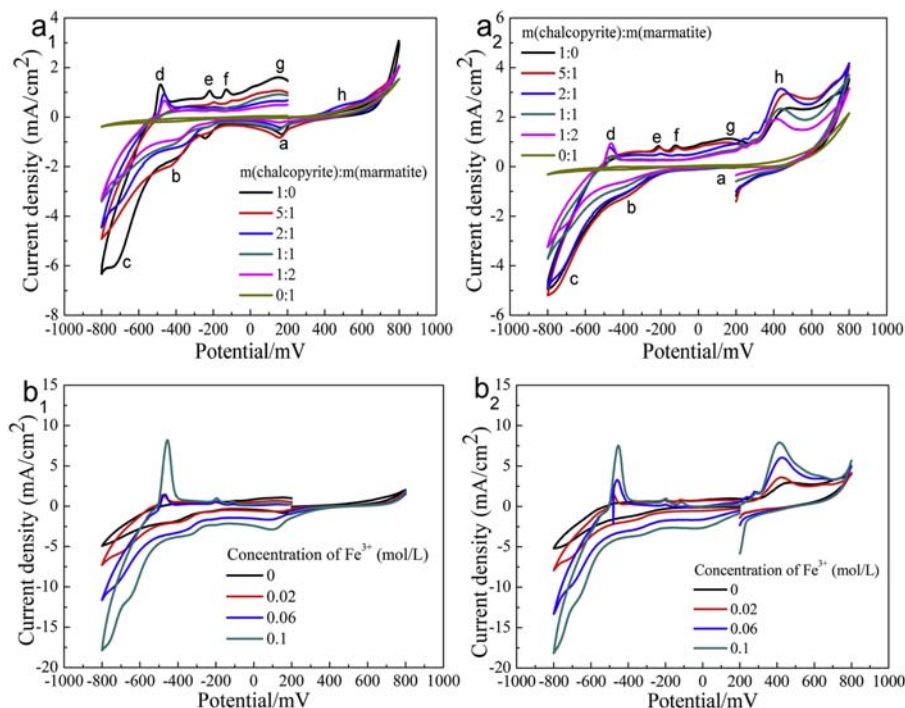


FIGURE 5.7 Cyclic voltammograms of chalcopyrite and marmatite in culture medium (pH 1.70). (A) Without Fe^{3+} ; (B) With Fe^{3+} (chalcopyrite to marmatite ratio of 5:1). From Zhao, H., Gan, X., Wang, J., Tao, L., Qin, W., & Qiu, G. (2017). Stepwise bioleaching of Cu–Zn mixed ores with comprehensive utilization of silver-bearing solid waste through a new technique process. *Hydrometallurgy*, 171, 374–386. <https://doi.org/10.1016/j.hydromet.2017.06.002>.

the oxidation reaction of marmatite, as shown in Eq. (5.1) (Jin-rong et al., 2013; Qin et al., 2012). Electrochemical interactions between chalcopyrite and marmatite did not significantly change their electrochemical dissolution process. Fig. 5.7(b₁–b₂) indicates that the presence of Fe^{3+} remarkably enhanced the oxidation-reduction rate of Cu–Zn mixed samples (chalcopyrite to marmatite ratio of 5:1), in accordance with the bioleaching results:

Fig. 5.8 shows the potentiostatic polarization of samples in the culture medium. Chalcopyrite alone could be directly oxidized when the applied potential was higher than 0.3 V (versus Ag/AgCl) and the marmatite alone could be oxidized when the applied potential exceeded 0.2 V (versus Ag/AgCl). The boundary value of the applied potential between the oxidation and reduction region of the Cu–Zn mixed sample (chalcopyrite to marmatite ratio of 5:1) was

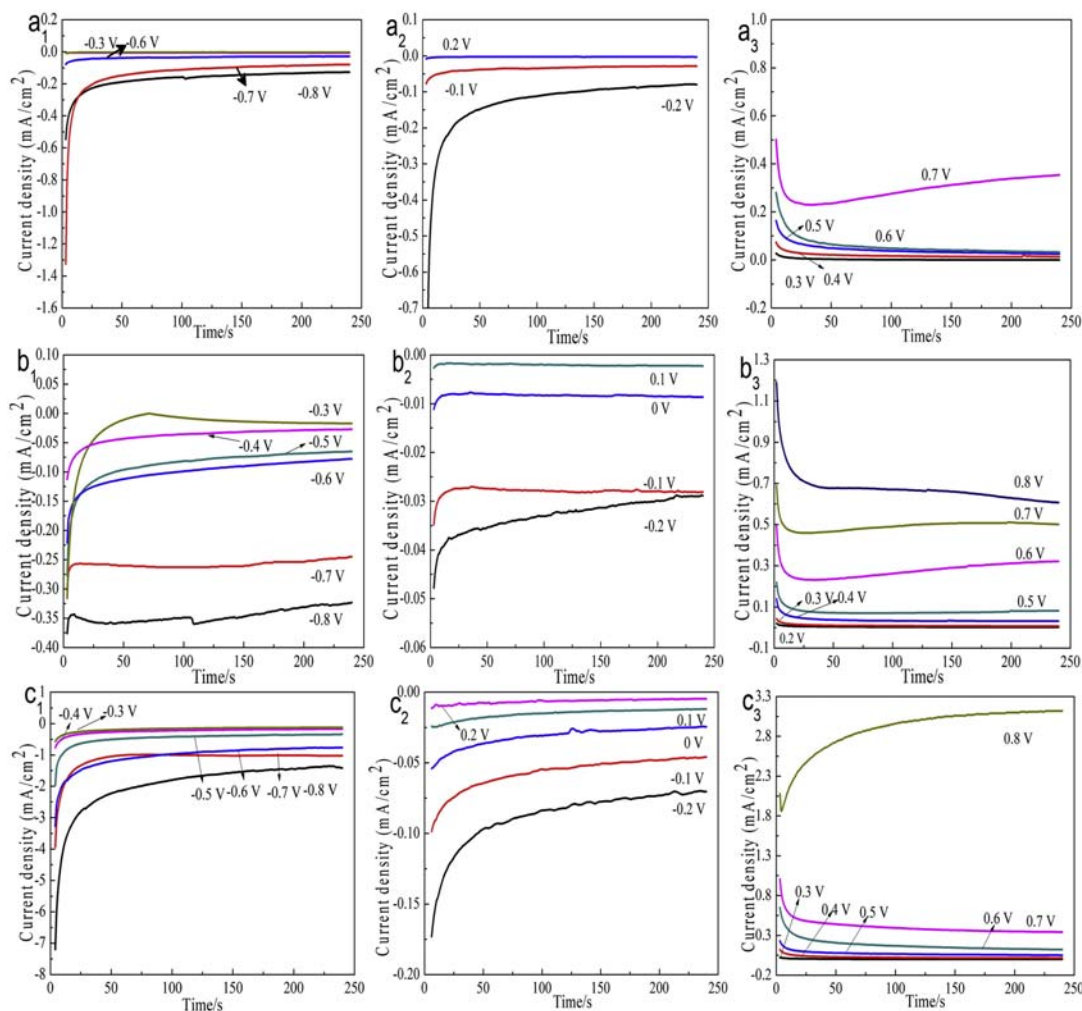


FIGURE 5.8 Potentiostatic polarization of samples in culture medium (pH 1.70). (A) Chalcopyrite alone; (B) Marmatite alone; (C) Cu–Zn mixed samples (chalcopyrite to marmatite ratio of 5:1). From Zhao, H., Gan, X., Wang, J., Tao, L., Qin, W., & Qiu, G. (2017). Stepwise bioleaching of Cu–Zn mixed ores with comprehensive utilization of silver-bearing solid waste through a new technique process. *Hydrometallurgy*, 171, 374–386. <https://doi.org/10.1016/j.hydromet.2017.06.002>.

0.3 V (versus Ag/AgCl). The relation between the total charges and applied potential in culture medium evaluated from the current–time curves is shown in Fig. 5.9. The oxidation rate of marmatite was higher than that of chalcopyrite, but the reduction rate of marmatite was lower than that of chalcopyrite and the electrochemical behavior of the Cu–Zn mixed sample (chalcopyrite to marmatite ratio of 5:1) was similar to that of chalcopyrite alone.

Fig. 5.10 shows the Tafel curves of samples with different ratios of chalcopyrite and marmatite in a culture medium without Fe^{3+} . The obtained values of the corrosion potential and corrosion current density are shown in Fig. 5.11. The corrosion potential (E_{corr}) of chalcopyrite alone is significantly higher than that of marmatite alone, and the corrosion potential of Cu–Zn mixed samples decreased with an increase in the marmatite ratio. Marmatite dissolved preferentially owing to its relatively low corrosion potential. Chalcopyrite and marmatite formed a galvanic cell in which chalcopyrite with a higher corrosion potential

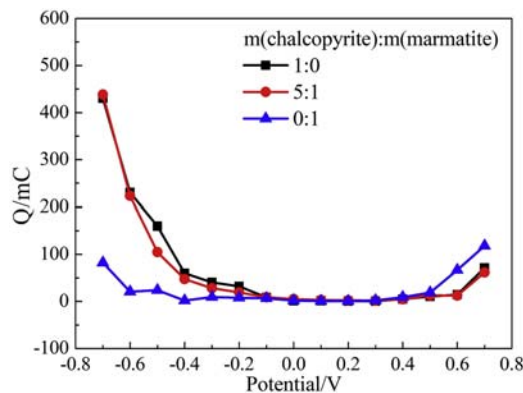


FIGURE 5.9 Relation between total charges (evaluated from current–time curves) and applied potential in culture medium. From Zhao, H., Gan, X., Wang, J., Tao, L., Qin, W., & Qiu, G. (2017). Stepwise bioleaching of Cu–Zn mixed ores with comprehensive utilization of silver-bearing solid waste through a new technique process. *Hydrometallurgy*, 171, 374–386. <https://doi.org/10.1016/j.hydromet.2017.06.002>.

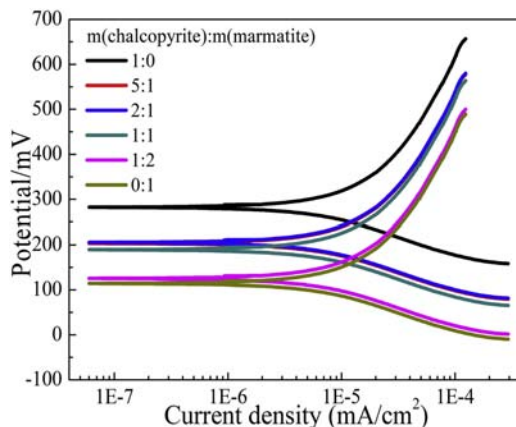


FIGURE 5.10 Tafel curves of samples with different ratios of chalcopyrite and marmatite in a culture medium (without Fe^{3+} addition). From Zhao, H., Gan, X., Wang, J., Tao, L., Qin, W., & Qiu, G. (2017). Stepwise bioleaching of Cu–Zn mixed ores with comprehensive utilization of silver-bearing solid waste through a new technique process. *Hydrometallurgy*, 171, 374–386. <https://doi.org/10.1016/j.hydromet.2017.06.002>.

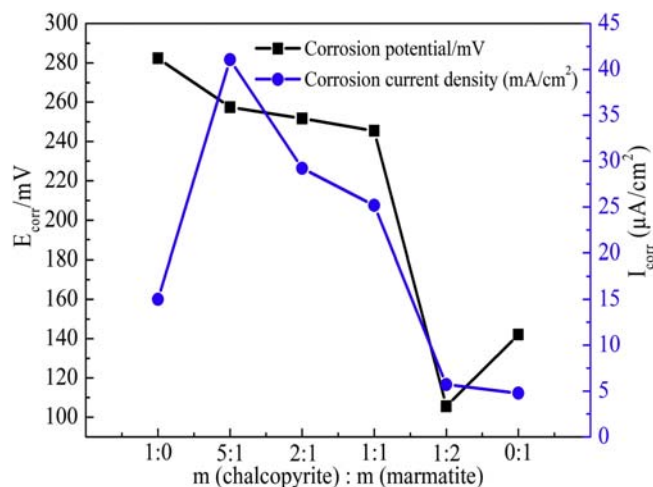


FIGURE 5.11 Corrosion potential and corrosion current density for samples with different ratios of chalcopyrite and marmatite. From Zhao, H., Gan, X., Wang, J., Tao, L., Qin, W., & Qiu, G. (2017). Stepwise bioleaching of Cu-Zn mixed ores with comprehensive utilization of silver-bearing solid waste through a new technique process. *Hydrometallurgy*, 171, 374–386. <https://doi.org/10.1016/j.hydromet.2017.06.002>.

acted as the cathodic part whose oxidation process was inhibited and marmatite with a lower corrosion potential acted as the anodic part whose oxidation process was enhanced. Except for the galvanic current, Cu^{2+} accelerated marmatite dissolution (Song et al., 2008). The result of the electrochemistry analysis is in accordance with bioleaching results. The higher corrosion current density (I_{corr}) of Cu-Zn mixed samples can be attributed to the galvanic current between chalcopyrite and marmatite.

Fig. 5.12 presents the Tafel curves of Cu-Zn mixed samples (chalcopyrite to marmatite ratio of 5:1) in a culture medium with different Fe^{3+} concentrations. The calculated values of E_{corr} and I_{corr} are provided in Fig. 5.13. The corrosion potential increased with an increase

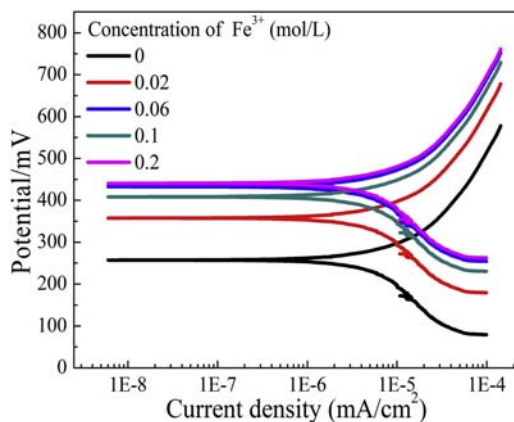


FIGURE 5.12 Tafel curves of Cu-Zn mixed samples (chalcopyrite to marmatite ratio of 5:1) in a culture medium with different Fe^{3+} concentrations. From Zhao, H., Gan, X., Wang, J., Tao, L., Qin, W., & Qiu, G. (2017). Stepwise bioleaching of Cu-Zn mixed ores with comprehensive utilization of silver-bearing solid waste through a new technique process. *Hydrometallurgy*, 171, 374–386. <https://doi.org/10.1016/j.hydromet.2017.06.002>.

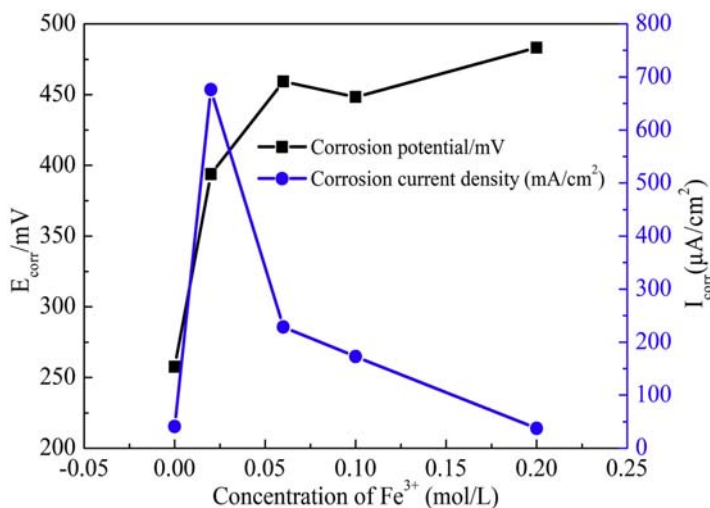


FIGURE 5.13 Corrosion potential and corrosion current density for Cu-Zn mixed samples (chalcopyrite to marmatite ratio of 5:1) in a culture medium with different Fe³⁺ concentrations. From Zhao, H., Gan, X., Wang, J., Tao, L., Qin, W., & Qiu, G. (2017). Stepwise bioleaching of Cu-Zn mixed ores with comprehensive utilization of silver-bearing solid waste through a new technique process. *Hydrometallurgy*, 171, 374–386. <http://doi.org/10.1016/j.hydromet.2017.06.002>.

in the Fe³⁺ concentration. The addition of Fe³⁺ increased the corrosion current density of Cu–Zn mixed samples (chalcopyrite to marmatite ratio of 5:1) and the Fe³⁺ concentration was appropriate to enhance the corrosion current density efficiently. This agrees well with the bioleaching results.

5.1.4 Cu and Ag recovery from bioleaching residue

The final bioleaching residues were subsequently leached by thiourea. Thiourea leaching experiments were carried out in 50-mL shake flasks at a liquid–solid ratio of 10:1, pH 1.0, and 45°C for 5.0 h. When zinc was almost totally extracted from marmatite, the liquid and solid were separated to obtain copper-bearing bioleaching residue. Silver-bearing ZLR was used to catalyze chalcopyrite bioleaching. Fig. 5.14 shows that adding ZLR remarkably accelerated the dissolution of chalcopyrite, and copper extraction increased from about 20% to more than 80%. In addition, the dissolution kinetics of Fe was enhanced owing to the addition of ZLR.

The final bioleaching residues were subsequently leached by thiourea. Thiourea leaching experiments were carried out in 50-mL shake flasks at a liquid–solid ratio of 10:1, pH 1.0, and 45°C for 5.0 h. A final silver extraction of about 90% was obtained through this process (Fig. 5.15). Cyanide leaching of silver from ores in alkaline solution using sodium cyanide has been used in industrial production for nearly a century. However, its industrial practice has been restricted because of serious contamination of the environment. Thiourea was considered to be one of the most promising alternative lixiviants because of its low toxicity and rapid kinetics in silver leaching. Moreover, thiourea has high selectivity to precious metals over base metals such as copper, which helped it to obtain commercial application

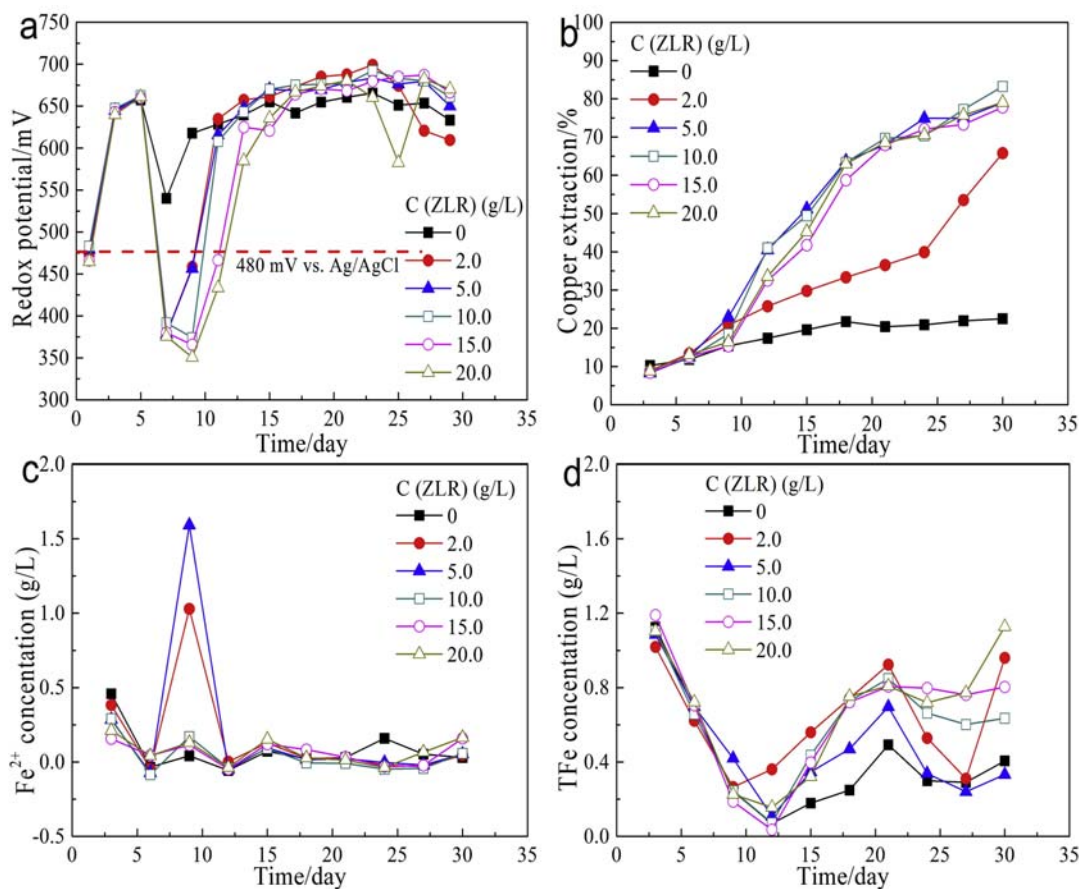


FIGURE 5.14 Catalytic bioleaching of chalcopyrite residue by zinc leaching residue (ZLR). From Zhao, H., Gan, X., Wang, J., Tao, L., Qin, W., & Qiu, G. (2017). Stepwise bioleaching of Cu–Zn mixed ores with comprehensive utilization of silver-bearing solid waste through a new technique process. *Hydrometallurgy*, 171, 374–386. <https://doi.org/10.1016/j.hydromet.2017.06.002>.

(Gurung et al., 2013). Thiourea leaching of silver should be conducted in an acidic solution because thiourea is unstable in an alkaline solution, so a low and stable pH is essential for effective thiourea leaching of silver. Here, acid-consuming components were consumed in the bioleaching process, which makes thiourea leaching of silver from bioleaching residues much more effective.

A technique for processing Cu–Zn mixed ores is proposed in Fig. 5.16. In the first step, noncatalyzed bioleaching of chalcopyrite–marmatite ore was used to produce a Zn Pregnant Leach Solution (PLS) and a first residue. Then, the catalyzed bioleaching of chalcopyrite by the addition of ZLR to the first residues was carried out to produce a Cu PLS and a second residue. In the third step, Ag recovery by the addition of thiourea to the second residue was implemented to make an Ag PLS and a final residue. Finally, recycling part of the recovered

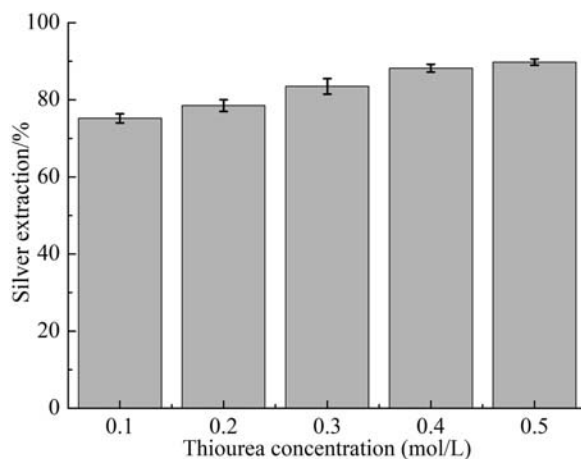


FIGURE 5.15 Thiourea leaching of final bioleaching residues to recover silver. From Zhao, H., Gan, X., Wang, J., Tao, L., Qin, W., & Qiu, G. (2017). Stepwise bioleaching of Cu-Zn mixed ores with comprehensive utilization of silver-bearing solid waste through a new technique process. *Hydrometallurgy*, 171, 374–386. <https://doi.org/10.1016/j.hydromet.2017.06.002>.

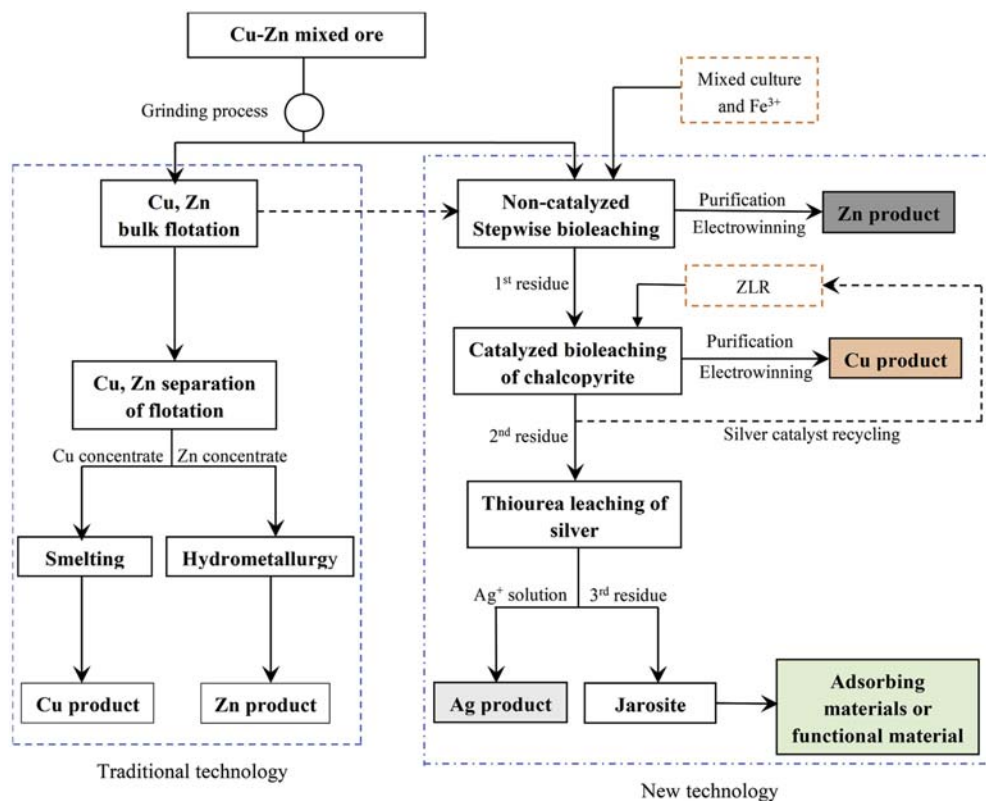


FIGURE 5.16 Proposed technique for Cu-Zn mixed ores. From Zhao, H., Gan, X., Wang, J., Tao, L., Qin, W., & Qiu, G. (2017). Stepwise bioleaching of Cu-Zn mixed ores with comprehensive utilization of silver-bearing solid waste through a new technique process. *Hydrometallurgy*, 171, 374–386. <https://doi.org/10.1016/j.hydromet.2017.06.002>.

Ag to the catalyzed bioleaching step can be considered to be carried out. The final residues consisted of jarosite are used as raw materials to prepare adsorbing materials or other functional materials. This technique accomplishes the stepwise dissolution of Cu–Zn mixed ores and the comprehensive use of silver-bearing ZLR.

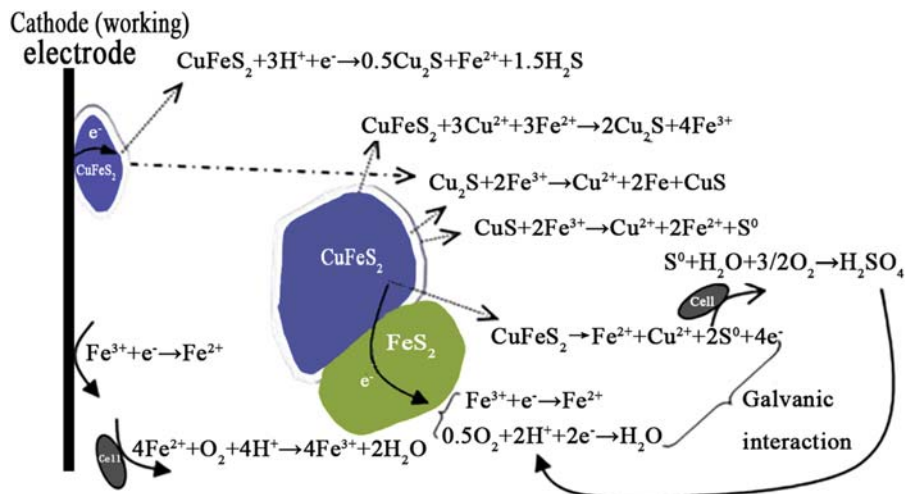
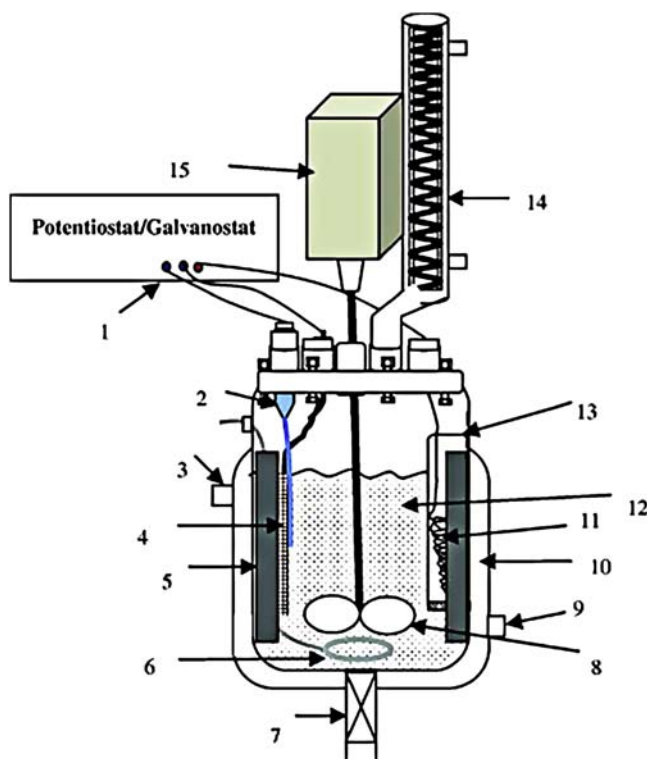
5.2 Role of pyrite in chalcopyrite bioleaching

Pyrite (FeS_2) is the most common sulfide mineral in nature (Aghazadeh et al., 2015; Wen et al., 2017). As a gangue, pyrite is frequently associated with other base metal sulfide minerals such as sphalerite, chalcopyrite, and galena (Zhang et al., 1997). Therefore, the role of pyrite in chalcopyrite bioleaching was extensively investigated.

5.2.1 Galvanic effect

Essentially, the galvanic effect is the basis of studying the interaction of sulfide minerals; it has been applied to several minerals in the industry (Saavedra et al., 2018). In the Acid Mine Drainage (AMD) or biohydrometallurgy systems, the galvanic effect occurs when different minerals make contact. The mineral with a higher open circuit current (OCP), or rest potential, functioning as the cathode is protected, whereas the mineral with lower potential acting as the anode is oxidized more quickly (Saavedra et al., 2018; Wang, Liao, Zhao et al., 2018; Wang, Chen, & Zhou, 2018).

Similarly, there is a galvanic effect between chalcopyrite and pyrite. Koleini et al. (Koleini et al., 2010) investigated the ferric sulfate leaching of chalcopyrite in the atmosphere. Results indicated that the leaching rate was low without the addition of pyrite and a passive layer blocked the surface of chalcopyrite particles. However, the oxidative dissolution of chalcopyrite was enhanced in the presence of pyrite. The OCPs of chalcopyrite and pyrite are 520 and 630 mV, respectively (versus Standard Hydrogen Electrode (SHE)), in 0.5 M sulfuric acid at 20°C; therefore, galvanic interaction between these two minerals occurred, and as the anode, the dissolution of chalcopyrite was promoted. More than 95% copper was leached in this work in less than 24 h, and the optimum experimental settings were a stirring speed of 1150 rpm, a solid weight percent of 2.5%, a total iron concentration of 5 g/L, a pyrite to chalcopyrite (5 g) ratio of 4, a solution potential of 410 mV (versus Ag/AgCl), a temperature of 85°C, and an initial sulfuric acid concentration of 45 g/L. In other work, Ahmadi et al. (2012) compared the effects of pyrite on chalcopyrite chemical leaching, bioleaching and electrobiochemical leaching. Results suggested that the leaching rate and yield of copper were improved in the presence of pyrite under the three conditions in which electrobiochemical leaching (Fig. 5.17) achieved the highest copper recovery (about 90%) after 10 days. The optimum experimental settings were a stirring speed of 600 rpm, solid weight percent of 20%, moderate thermophiles with Norris nutrient medium and 0.02% (w/w) yeast extract addition, pyrite to chalcopyrite ratio of 1, solution potential of 410–430 mV (versus Ag/AgCl), temperature of 50°C, and initial pH of 1.5. The authors proposed that pyrite was inert under a low potential condition; it enhanced the anodic dissolution of chalcopyrite. Detailed interactions are shown in Fig. 5.18. Similarly, Yi et al. (2016) reported that the galvanic dissolution of chalcopyrite was preferential in chemical leaching (150 mL 9K basic salt media with 2.25 g/L Fe^{2+} and 0.75 g/L Fe^{3+} , 1.2 g chalcopyrite and 3.6 g pyrite, 30 or 48°C) as the



solution potentials fluctuated at a low range (320–420 mV versus Ag/AgCl), but chalcopyrite dissolution was inhibited when microbes were inoculated at 30°C because of the high potential resulting from the oxidation of Fe^{2+} . Yang et al. also suggested that no negative impact occurred on the galvanic leaching of chalcopyrite in the presence of microbes at 48°C owing to the high reaction kinetics at a higher temperature.

To explore the galvanic effect directly between pyrite and chalcopyrite, electrochemical experiments were conducted. Majuste et al. (2012) compared the OCPs (or mixed potentials [E_M]) and dissolution current densities (i_{diss}) of chalcopyrite electrodes respectively containing 0% (CE), 14% (ME1), 31% (ME2), and 42% (ME3) pyrite. For comparison, a pure pyrite electrode (PE) was also researched. The detected OCPs (E_M) (Table 5.2) and i_{diss} (Table 5.3) under different oxidizing conditions were both positively correlated with the pyrite content, which indicated that the dissolution of chalcopyrite was promoted by pyrite. In addition, results suggested that the promotion effect of pyrite on chalcopyrite dissolution was more significant under the less oxidizing condition (only residual oxygen after nitrogen bubbling), because the electrode reactions were barely impeded by the formed surface passivation substances compared with the high oxidizing conditions (with oxygen and ferric ions as oxidants). Nevertheless, the absolute i_{diss} of chalcopyrite under the less oxidizing condition was limited. Olvera et al. (2014) also suggested that the galvanic effect between chalcopyrite and pyrite

TABLE 5.2 E_M (open circuit current) (V/SHE) for different electrodes in 0.1 mol/L H_2SO_4 media after nitrogen bubbling under different oxidizing conditions (Majuste et al., 2012).

Electrode	E_M	E_M (0.001 M O_2)	E_M (0.01 M Fe^{3+})	E_M (0.05 M Fe^{3+})	E_M (0.001 M O_2 , 0.05 M Fe^{3+})
Chalcopyrite electrode	0.47 ± 0.02	0.55 ± 0.01	0.63 ± 0.01	0.68 ± 0.01	0.72 ± 0.02
ME1	0.55 ± 0.02	0.58 ± 0.01	0.71 ± 0.01	0.75 ± 0.01	0.78 ± 0.02
ME2	0.57 ± 0.01	0.61 ± 0.01	0.72 ± 0.01	0.77 ± 0.01	0.81 ± 0.01
ME3	0.59 ± 0.01	0.63 ± 0.01	0.74 ± 0.01	0.78 ± 0.01	0.82 ± 0.01
Pyrite electrode	0.61 ± 0.02	0.70 ± 0.01	0.78 ± 0.01	0.81 ± 0.01	0.85 ± 0.01

TABLE 5.3 Dissolution current densities ($\mu\text{A}/\text{cm}^2$) for mineral electrodes in 0.1-mol/L H_2SO_4 solutions after nitrogen bubbling under different oxidizing conditions (Majuste et al., 2012).

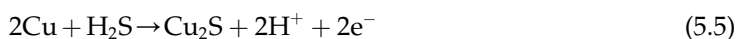
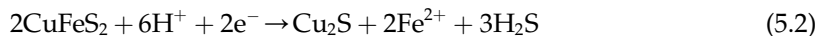
Electrode	i_{diss}	i_{diss} (0.001 M O_2)	i_{diss} (0.01 M Fe^{3+})	i_{diss} (0.05 M Fe^{3+})	i_{diss} (0.001 M O_2 , 0.05 M Fe^{3+})
Chalcopyrite electrode	0.26 ± 0.08	4.80 ± 0.49	8.00 ± 0.88	9.33 ± 0.97	14.55 ± 0.96
ME1	4.00 ± 0.38	5.87 ± 0.49	11.19 ± 1.25	14.94 ± 1.10	18.48 ± 1.31
ME2	6.32 ± 0.62	8.75 ± 0.71	12.74 ± 1.24	17.43 ± 1.46	20.72 ± 1.55
ME3	10.40 ± 0.94	12.23 ± 0.97	16.73 ± 1.46	20.09 ± 1.43	23.66 ± 1.94

was impeded owing to the passive film formed on the chalcopyrite surface causing the low diffusion rates of ions and transfer rates of electrons. Therefore, a silver-enhanced pyrite was used in another work to achieve a dramatic improvement in chalcopyrite dissolution, in which the introduced silver might react with passivation substances (such as elemental sulfur), causing the formation of the more conductive silver sulfide. Although the recovery of silver from the leaching system was difficult, recycling of silver-enhanced pyrite for further leaching was available (Nazari et al., 2012a, 2012b).

5.2.2 Mediating the oxidation-reduction potential (reduction mechanism)

Aside from the galvanic effect, other solution conditions strongly act on the dissolution of mixed ores. Conversely, these conditions are also affected by mineral dissolution (Jafari et al., 2019). For example, the introduction of pyrite promotes chalcopyrite dissolution by deflecting the ORP into its optimal range. The succession and density of microorganisms in chalcopyrite bioleaching systems were changed by adding pyrite (Wang et al., 2018; Xiao et al., 2017), and pyrite also caused a decline in pH levels and an increase in iron ion concentrations during chalcopyrite bioleaching (Ahmadi et al., 2012). To some extent, these elements can be integrated into the concept of ORP, which is a significant factor in relation to the thermodynamic feasibility and kinetic rate of oxidation dissolution. This statement can be proved through comprehensive research on chalcopyrite bioleaching: maintaining the concentration ratio of $\text{Fe}^{3+}/\text{Fe}^{2+}$ within a suitable range by controlling the rate of biological oxidation of ferrous (or by adding Fe^{3+} and Fe^{2+} substances directly), to regulate the ORP (Christel et al., 2018; Masaki et al., 2018).

For chalcopyrite dissolution, the optimum potential interval was commonly discussed (Zhao et al., 2019). Chalcopyrite was reported to dissolve quickly under lower potential conditions because the more soluble Cu_2S might form as the reduction product of chalcopyrite (Eqs. 5.2–5.5). However, when the solution potential was maintained at the high levels, the chalcopyrite surface was blocked by the intermediate passive substance layer, impeding the dissolution rate (Hiroyoshi et al., 2000; Vilcáez et al., 2009; Zhao, Wang, Gan et al., 2015; Zhao, Wang, Yang et al., 2015). Therefore, various methods, such as adding chemical reagents, adjusting the dissolved oxygen, using electrochemical reactors, adjusting the microorganisms, and adding other sulfide minerals, were developed to control the solution potential in the corresponding ranges (Zhao et al., 2019):



Although the existence of the optimum potential interval was extensively approved, the absolute threshold values varied in different reports. To solve this problem, Zhao, Wang, Gan et al. (2015), Zhao, Wang, Yang et al. (2015) established a mathematic model to predict

the optimum redox potential in chalcopyrite leaching. According to the Gibbs free energy function and Nernst equation, the ΔG of Eq. (5.2) can be calculated as shown in Eq. (5.6). Then, the ORP of the bioleaching system is determined by the ratio of Fe^{3+} to Fe^{2+} , resulting in Eq. (5.7), where G is the Gibbs free energy, R is the gas constant (8.314 J/molK), T is the absolute temperature (318K), F is the Faraday constant (96,485 C/mol), n is the number of charge transfer, and E is the potential of the reaction. Combining Eqs. (5.6) and (5.7), the critical potential (E_{H1}) of chalcopyrite reduction reaction by Eq. (5.2) is shown in Eq. (5.8). Chalcopyrite can be reduced when the solution potential is lower than this critical potential; therefore, it is called E_H , which is a function of the concentrations of Cu^{2+} and Fe^{2+} . In the same way, Eqs. (5.9) and (5.10) respectively show the ΔG and the critical potential (E_{H2}) of chalcopyrite reduction reaction in Eq. (5.3). The values of E_{H1} and E_{H2} are similar, so the smaller one (E_{H2}) is adopted as the maximum threshold (E_H) of the optimum potential range. For the further oxidation of the formed Cu_2S shown in Eqs. (5.4) and (5.5), the corresponding ΔG are respectively shown in Eqs. (5.11) and (5.12), and the corresponding critical potentials (E_{L1} and E_{L2}) are respectively shown in Eqs. (5.13) and (5.14). E_{L1} and E_{L2} are both related to the concentration of Cu^{2+} . Therefore, the greater one (E_{L2}) should be adopted as the minimum threshold (E_L) of the optimum potential range. The calculated optimum potential range is thus E_L (Eq. 5.14) to E_H (Eq. 5.8):

$$\text{H}_2\text{S} \rightarrow \text{S}^0 + 2\text{H}^+ + 2\text{e}^- \quad (5.6)$$

$$\text{Cu}_2\text{S} \rightarrow \text{Cu}_x\text{S} + (2-x)\text{Cu}^2 + (4-2x)\text{e}^- \quad (5.7)$$

$$\text{CuFeS}_2 \rightarrow \text{Cu}_{1-x}\text{Fe}_{1-y}\text{S}_{2-z} + x\text{Cu}^{2+} + y\text{Fe}^{2+} + z\text{S}^0 + 2(x+y)\text{e}^- \quad (5.8)$$

$$\Delta G = \Delta G^0 + RT \ln \frac{(a\text{Fe}^{2+})}{(a\text{Cu}^{2+})^3} = -nEF \quad (5.9)$$

$$E_{H2} = 481 + 47.3 \lg(a\text{Cu}^{2+}) - 15.77 \lg(a\text{Fe}^{2+}) / \text{mV} \quad (5.10)$$

$$\Delta G = \Delta G^0 + RT \ln \frac{(a\text{Fe}^{2+})^4 (a\text{Cu}^{2+})^2}{(a\text{Fe}^{3+})^4} = -nEF \quad (5.11)$$

$$\Delta G = \Delta G^0 + RT \ln (a\text{Cu}^{2+})^4 = -nEF \quad (5.12)$$

$$E_{L1} = 362 + 31.5 \lg(a\text{Cu}^{2+}) / \text{mV} \quad (5.13)$$

$$E_{L1} = 401 + 29.5 \lg(a\text{Cu}^{2+}) / \text{mV} \quad (5.14)$$

Subsequently, Zhao, Wang, Gan et al. (2015), Zhao, Wang, Yang et al. (2015) investigated the effect of pyrite on chalcopyrite dissolution in the presence of the moderately thermophilic

iron-oxidizing bacteria *L. ferriphilum* (45°C). Results showed that the redox potentials were deduced and maintained between the proposed optimum range of 380–480 mV versus Ag/AgCl after the introduction of pyrite, and a copper extraction of about 80%–95% was obtained.

In addition, in terms of surface products, [Nazari and Asselin \(2009\)](#) proposed that iron ions were preferentially released over copper during chalcopyrite dissolution in the absence of pyrite, forming an iron-deficient copper polysulfide passivating layer. On the other hand, the existence of pyrite could provide a catalytic surface area for the reduction of excess Fe^{3+} , mediating the ORP in the optimum range. Moreover, sulfur ions on a chalcopyrite surface tended to be converted to S_8 rings with a highly porous morphology in the optimum potential range, which could not inhibit the transfer of reactants and products, and thus promoted the further dissolution of chalcopyrite.

5.3 Other gangue minerals

In addition to sulfide minerals such as marmatite and pyrite, the effect of bornite on chalcopyrite dissolution was studied by several researchers. [Zhao et al. \(2013\)](#), [Zhao, Wang, Gan et al. \(2015\)](#), [Zhao, Wang, Yang et al. \(2015\)](#) proposed that a synergistic effect between chalcopyrite and bornite was achieved during bioleaching by moderately thermophilic bacteria (*L. ferriphilum*) at 45°C and by mesophilic bacteria (*Acidithiobacillus ferrooxidans*) at 30°C. Unlike pyrite, the OCP of bornite was less than that of chalcopyrite; therefore, the galvanic effect promoted the anodic dissolution of bornite. Moreover, the solution potential could be maintained at the optimum range (370–480 mV versus Ag/AgCl) after the addition of bornite. As the cathode, chalcopyrite was more easily reduced, and thus the reduction mechanism occurred. The interaction mechanism between chalcopyrite and bornite in bioleaching is shown as [Fig. 5.19](#).

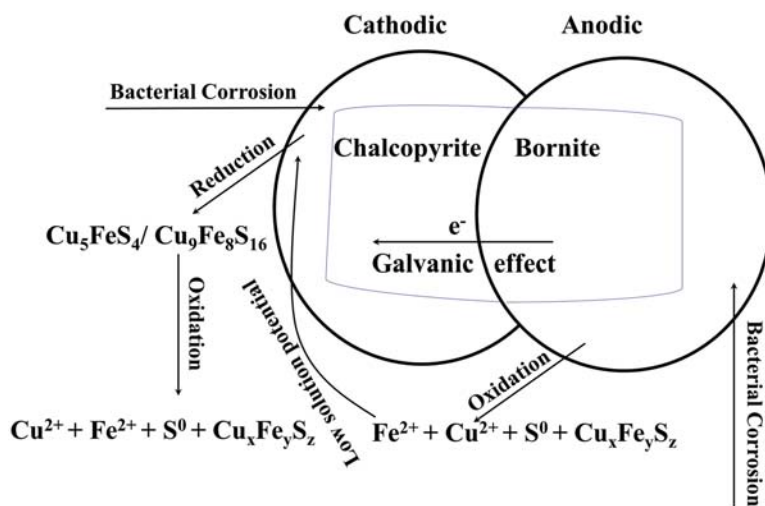


FIGURE 5.19 Model of interaction between chalcopyrite and bornite in bioleaching. From Zhao, H., Wang, J., Hu, M., Qin, W., Zhang, Y., & Qiu, G. (2013). Synergistic bioleaching of chalcopyrite and bornite in the presence of *Acidithiobacillus ferrooxidans*. *Bioresource Technology*, 149, 71–76. <https://doi.org/10.1016/j.biortech.2013.09.035>.

Conventionally, raw chalcopyrite ores are primarily processed by flotation to acquire a chalcopyrite concentrate. Because sulfide ores and oxide ores or silicates or carbonates have obvious differences in hydrophobicity, it is easy to separate them by flotation methods. Sulfides such as sphalerite (marmatite), pyrite, and bornite are the main components of chalcopyrite concentrates and therefore are widely studied. However, low-grade copper deposits with large reserves cannot be treated efficiently by flotation. As an alternative technology, large-scale bioleaching methods such as heap leaching have become more promising. Therefore, in addition to sulfide ores, the influence of oxide ores, silicates, and carbonates on the dissolution of chalcopyrite also needs attention.

The typical oxide ore, quartz mineral (50 g/L), was reported to accelerate the bioleaching of chalcopyrite (2% wt/vol) in the presence of *A. ferrooxidans* at 30°C, because the passivation layer was removed from the chalcopyrite surface by mechanical friction (Dong et al., 2013). Compared with quartz, the effect of silicate minerals on chalcopyrite dissolution is more complicated owing to its soluble properties. When the solution pH value was lower than 2, more silicate minerals (microcline, plagioclase, muscovite, etc.) were dissolved, causing the consumption of acid and the promotion of solution viscosity; chalcopyrite dissolution was therefore inhibited (Dopson et al., 2008). Moreover, the dissolution of silicate minerals might liberate some toxic trace elements, such as fluoride, into the leaching system and impair the growth of microorganisms (Dopson et al., 2008). HF was considered to be the main toxic fluoride species, although it could react with Fe^{3+} and be converted to FeF_2^+ and FeF_2^- when the ratio of Fe^{3+} to F^- was greater than 1:1, and therefore the toxicity of F^- was weakened. Based on this, Ma et al. (2019) proposed that the bioleaching of chalcopyrite could be constructed by adding Fe^{3+} stepwise and maintaining the ratio of Fe^{3+} to F^- at about 6:1. If the ratio was too small, the toxicity of F^- could not be effectively impeded; in contrast, excess Fe^{3+} could induce the formation of jarosite, inhibiting chalcopyrite dissolution. Except for severe toxic ions such as F^- , other metallic ions such as Mg^{2+} , which were derived from the carbonate gangues dolomite and magnesite or other silicate minerals, released during gangue dissolution, could harm the activity of microorganisms and the bioleaching of chalcopyrite. Liu et al. (2015) reported that a inhibition of chalcopyrite bioleaching occurred when the Mg^{2+} concentration was greater than 20 g/L.

5.4 Summary

Stepwise bioleaching was feasible for Cu–Zn mixed ores of chalcopyrite and marmatite, especially for mixed ores consisting of a high mass ratio of chalcopyrite and a low mass ratio of marmatite. Marmatite was preferentially dissolved owing to its low corrosion potential. Electrochemical interactions between chalcopyrite and marmatite did not significantly change the electrochemical dissolution process, but accelerated marmatite oxidation and dissolution. A high redox potential should be maintained to make marmatite preferentially dissolve and accomplish stepwise bioleaching, which is why a bioleaching system of mixed culture with Fe^{3+} was the most effective for the stepwise bioleaching of Cu–Zn mixed ores. However, the intermediate species and dissolution processes of Cu–Zn mixed samples in the four different bioleaching systems were similar. Silver-bearing ZLR effectively promoted chalcopyrite dissolution in the catalytic bioleaching of copper-containing bioleaching residue.

The presence of ZLR changed the previous dissolution mechanism of chalcopyrite and chalcopyrite bioleaching was no longer determined by the redox potential. The final bioleaching residues were subsequently leached by thiourea to recover silver efficiently. The proposed three-step technique process for Cu–Zn mixed ores is a promising technique for effectively processing Cu–Zn mixed ores containing chalcopyrite and marmatite and for the comprehensive use of silver-bearing solid waste. Regarding the effect of pyrite on chalcopyrite dissolution, the proposed optimum potential range mechanism (the reduction mechanism) was important. Pyrite could deduce or promote the solution potential to the optimum range in the presence and absence, respectively, of microorganisms. Pyrite might dissolve quickly in biotic systems and release massive Fe^{2+} , causing a decrease in the potential; on the contrary, relatively small amounts of Fe^{2+} were liberated in abiotic systems (with no additional iron ions), which were further oxidized by oxygen, resulting in promotion of the potential. On the other hand, the galvanic effect undoubtedly had a crucial role. Moreover, the galvanic effect might interact with the reduction mechanism to dissolve chalcopyrite, because the lower potential was more effective for both of them; this was confirmed by Esmailbagi et al. (2018). Therefore, a hypothesis was proposed that the galvanic effect and the reduction mechanism acted on the dissolution of chalcopyrite simultaneously, whereas the former occurred when chalcopyrite and pyrite were in contacted with each other and the latter affected the chalcopyrite particles individually. In addition to quartz, other silicate and carbonate gangues seem to cause trouble in the bioleaching of chalcopyrite. Therefore, solutions aiming at removing these gangues beforehand, promoting the metallic ion tolerance of microorganisms, and controlling the released toxic ions need to be studied.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (51704331), Young Elite Scientists Sponsorship Program by CAST (2017QNRC001), National Basic Research Program of China (Grant No. 2010CB630905), and High-Tech Research and Development Program of China (Grant No. 2012AA061501).

References

- Aghazadeh, S., Mousavinezhad, S. K., & Gharabaghi, M. (2015). Chemical and colloidal aspects of collectorless flotation behavior of sulfide and non-sulfide minerals. *Advances in Colloid and Interface Science*, 225, 203–217. <https://doi.org/10.1016/j.cis.2015.09.007>.
- Ahmadi, A., Ranjbar, M., & Schaffie, M. (2012). Catalytic effect of pyrite on the leaching of chalcopyrite concentrates in chemical, biological and electrobiochemical systems. *Minerals Engineering*, 34, 11–18. <https://doi.org/10.1016/j.mineng.2012.03.022>.
- Arce, E. M., & González, I. (2002). A comparative study of electrochemical behavior of chalcopyrite, chalcocite and bornite in sulfuric acid solution. *International Journal of Mineral Processing*, 67(1), 17–28. [https://doi.org/10.1016/S0301-7516\(02\)00003-0](https://doi.org/10.1016/S0301-7516(02)00003-0).
- Boulton, A., Fornasiero, D., & Ralston, J. (2003). Characterisation of sphalerite and pyrite flotation samples by XPS and ToF-SIMS. *International Journal of Mineral Processing*, 70(1–4), 205–219. [https://doi.org/10.1016/S0301-7516\(03\)00003-6](https://doi.org/10.1016/S0301-7516(03)00003-6).
- Buckley, A. N., & Woods, R. (1984). An X-Ray photoelectron spectroscopic study of the oxidation of chalcopyrite. *Australian Journal of Chemistry*, 37(12), 2403–2413. <https://doi.org/10.1071/CH9842403>.
- Christel, S., Herold, M., Bellenberg, S., Buetti-Dinh, A., Hajjani, M. E., Pivkin, I. V., et al. (2018). Weak iron oxidation by *Sulfobacillus thermosulfidooxidans* maintains a favorable redox potential for chalcopyrite bioleaching. *Frontiers in Microbiology*, 9. <https://doi.org/10.3389/fmicb.2018.03059>.

- De Oliveira, C., De Lima, G. F., De Abreu, H. A., & Duarte, H. A. (2012). Reconstruction of the chalcopyrite surfaces-A DFT study. *Journal of Physical Chemistry C*, 116(10), 6357–6366. <https://doi.org/10.1021/jp300713z>.
- Dixon, D. G., Mayne, D. D., & Baxter, K. G. (2008). GalvanoxTM – a novel galvanically-assisted atmospheric leaching technology for copper concentrates. *Canadian Metallurgical Quarterly*, 47(3), 327–336. <https://doi.org/10.1179/cm.2008.47.3.327>. Metallurgical Society of CIM.
- Dong, Y. B., Lin, H., Zhou, S., Xu, X., & Zhang, Y. (2013). Effects of quartz addition on chalcopyrite bioleaching in shaking flasks. *Minerals Engineering*, 46–47, 177–179. <https://doi.org/10.1016/j.mineng.2013.04.014>.
- Dopson, M., Halinen, A. K., Rahunen, N., Boström, D., Sundkvist, J. E., Riekkola-Vanhanen, M., et al. (2008). Silicate mineral dissolution during heap bioleaching. *Biotechnology and Bioengineering*, 99(4), 811–820. <https://doi.org/10.1002/bit.21628>.
- Esmailbagi, M. R., Schaffie, M., Kamyabi, A., & Ranjbar, M. (2018). Microbial assisted galvanic leaching of chalcopyrite concentrate in continuously stirred bioreactors. *Hydrometallurgy*, 180, 139–143. <https://doi.org/10.1016/j.hydromet.2018.07.020>.
- Ghahremaninezhad, A., Asselin, E., & Dixon, D. G. (2010). Electrochemical evaluation of the surface of chalcopyrite during dissolution in sulfuric acid solution. *Electrochimica Acta*, 55(18), 5041–5056. <https://doi.org/10.1016/j.electacta.2010.03.052>.
- Ghahremaninezhad, A., Dixon, D. G., & Asselin, E. (2013). Electrochemical and XPS analysis of chalcopyrite (CuFeS₂) dissolution in sulfuric acid solution. *Electrochimica Acta*, 87, 97–112. <https://doi.org/10.1016/j.electacta.2012.07.119>.
- Gu, Guohua, Hu, K., Zhang, X., Xiong, X., & Yang, H. (2013). The stepwise dissolution of chalcopyrite bioleached by *Leptospirillum ferriphilum*. *Electrochimica Acta*, 103, 50–57. <https://doi.org/10.1016/j.electacta.2013.04.051>.
- Gurung, M., Adhikari, B. B., Kawakita, H., Ohto, K., Inoue, K., & Alam, S. (2013). Recovery of gold and silver from spent mobile phones by means of acidothiurea leaching followed by adsorption using biosorbent prepared from persimmon tannin. *Hydrometallurgy*, 133, 84–93. <https://doi.org/10.1016/j.hydromet.2012.12.003>.
- Harmer, S. L., Goncharova, L. V., Kolarova, R., Lennard, W. N., Muñoz-Márquez, M. A., Mitchell, I. V., et al. (2007). Surface structure of sphalerite studied by medium energy ion scattering and XPS. *Surface Science*, 601(2), 352–361. <https://doi.org/10.1016/j.susc.2006.10.001>.
- Harmer, S. L., Mierczynska-Vasilev, A., Beattie, D. A., & Shapter, J. G. (2008). The effect of bulk iron concentration and heterogeneities on the copper activation of sphalerite. *Minerals Engineering*, 21(12–14), 1005–1012. <https://doi.org/10.1016/j.mineng.2008.02.014>.
- Harmer, S. L., Thomas, J. E., Fornasiero, D., & Gerson, A. R. (2006). The evolution of surface layers formed during chalcopyrite leaching. *Geochimica et Cosmochimica Acta*, 70(17), 4392–4402. <https://doi.org/10.1016/j.gca.2006.06.1555>.
- Hiro Yoshi, N., Miki, H., Hirajima, T., & Tsunekawa, M. (2000). Model for ferrous-promoted chalcopyrite leaching. *Hydrometallurgy*, 57(1), 31–38. [https://doi.org/10.1016/S0304-386X\(00\)00089-X](https://doi.org/10.1016/S0304-386X(00)00089-X).
- Jafari, M., Abdollahi, H., Shafaei, S. Z., Gharabaghi, M., Jafari, H., Akcil, A., et al. (2019). Acidophilic bioleaching: A review on the process and effect of organic–inorganic reagents and materials on its efficiency. *Mineral Processing and Extractive Metallurgy Review*, 40(2), 87–107. <https://doi.org/10.1080/08827508.2018.1481063>.
- Jin-rong, B., Guo-hua, G., & Ke-ting, H. (2013). Bioleaching and electrochemical property of marmatite by *Leptospirillum ferrooxidans*. *Transactions of Nonferrous Metals Society of China*, 494–500. [https://doi.org/10.1016/s1003-6326\(13\)62490-5](https://doi.org/10.1016/s1003-6326(13)62490-5).
- Khoshkhoo, M., Dopson, M., Shchukarev, A., & Sandström, Å. (2014). Chalcopyrite leaching and bioleaching: An X-ray photoelectron spectroscopic (XPS) investigation on the nature of hindered dissolution. *Hydrometallurgy*, 149, 220–227. <https://doi.org/10.1016/j.hydromet.2014.08.012>.
- Klauber, C. (2008). A critical review of the surface chemistry of acidic ferric sulphate dissolution of chalcopyrite with regards to hindered dissolution. *International Journal of Mineral Processing*, 86(1–4), 1–17. <https://doi.org/10.1016/j.minpro.2007.09.003>.
- Klauber, C., Parker, A., Van Bronswijk, W., & Watling, H. (2001). Sulphur speciation of leached chalcopyrite surfaces as determined by x-ray photoelectron spectroscopy. *International Journal of Mineral Processing*, 62(1–4), 65–94. [https://doi.org/10.1016/S0301-7516\(00\)00045-4](https://doi.org/10.1016/S0301-7516(00)00045-4).
- Koleini, S. M. J., Jafarian, M., Abdollahy, M., & Aghazadeh, V. (2010). Galvanic leaching of chalcopyrite in atmospheric pressure and sulfate media: Kinetic and surface studies. *Industrial and Engineering Chemistry Research*, 49(13), 5997–6002. <https://doi.org/10.1021/ie100017u>.
- Konishi, Y., Asai, S., Tokushige, M., & Suzuki, T. (1999). Kinetics of the bioleaching of chalcopyrite concentrate by acidophilic thermophile *Acidianus brierleyi*. *Biotechnology Progress*, 15(4), 681–688. <https://doi.org/10.1021/bp9900662>.

- Li, Y., Kawashima, N., Li, J., Chandra, A. P., & Gerson, A. R. (2013). A review of the structure, and fundamental mechanisms and kinetics of the leaching of chalcopyrite. *Advances in Colloid and Interface Science*, 197–198, 1–32. <https://doi.org/10.1016/j.cis.2013.03.004>.
- Liang, C. L., Xia, Jin-Lan, Yang, Y., Nie, Z. Y., et al. (2011). Characterization of the thermo-reduction process of chalcopyrite at 65 °C by cyclic voltammetry and XANES spectroscopy. *Hydrometallurgy*, 107(1), 13–21. <https://doi.org/10.1016/j.hydromet.2011.01.011>.
- Liu, X., Wen, J. K., Wu, B., & Liu, S. (2015). Magnesium-rich gangue dissolution in column bioleaching of chalcopyrite. *Rare Metals*, 34(5), 366–370. <https://doi.org/10.1007/s12598-014-0445-6>.
- Majuste, D., Ciminelli, V. S. T., Osseo-Asare, K., & Dantas, M. S. S. (2012). Quantitative assessment of the effect of pyrite inclusions on chalcopyrite electrochemistry under oxidizing conditions. *Hydrometallurgy*, 113–114, 167–176. <https://doi.org/10.1016/j.hydromet.2011.12.020>.
- Masaki, Y., Hirajima, T., Sasaki, K., Miki, H., & Okibe, N. (2018). Microbiological redox potential control to improve the efficiency of chalcopyrite bioleaching. *Geomicrobiology Journal*, 35(8), 648–656. <https://doi.org/10.1080/01490451.2018.1443170>.
- Ma, L., Wu, J., Liu, X., Tan, L., & Wang, X. (2019). The detoxification potential of ferric ions for bioleaching of the chalcopyrite associated with fluoride-bearing gangue mineral. *Applied Microbiology and Biotechnology*, 103(5), 2403–2412. <https://doi.org/10.1007/s00253-018-09599-6>.
- Nakai, I., Sugitani, Y., Nagashima, K., & Niwa, Y. (1978). X-ray photoelectron spectroscopic study of copper minerals. *Journal of Inorganic and Nuclear Chemistry*, 40(5), 789–791. [https://doi.org/10.1016/0022-1902\(78\)80152-3](https://doi.org/10.1016/0022-1902(78)80152-3).
- Nazari, G., & Asselin, E. (2009). Morphology of chalcopyrite leaching in acidic ferric sulfate media. *Hydrometallurgy*, 96(3), 183–188. <https://doi.org/10.1016/j.hydromet.2008.09.004>.
- Nazari, G., Dixon, D. G., & Dreisinger, D. B. (2011). Enhancing the kinetics of chalcopyrite leaching in the GalvanoxTM process. *Hydrometallurgy*, 105(3–4), 251–258. <https://doi.org/10.1016/j.hydromet.2010.10.013>.
- Nazari, G., Dixon, D. G., & Dreisinger, D. B. (2012a). The mechanism of chalcopyrite leaching in the presence of silver-enhanced pyrite in the GalvanoxTM process. *Hydrometallurgy*, 113–114, 122–130. <https://doi.org/10.1016/j.hydromet.2011.12.011>.
- Nazari, G., Dixon, D. G., & Dreisinger, D. B. (2012b). The role of galena associated with silver-enhanced pyrite in the kinetics of chalcopyrite leaching during the GalvanoxTM process. *Hydrometallurgy*, 111–112(1), 35–45. <https://doi.org/10.1016/j.hydromet.2011.09.011>.
- Olvera, O. G., Quiroz, L., Dixon, D. G., & Asselin, E. (2014). Electrochemical dissolution of fresh and passivated chalcopyrite electrodes. Effect of pyrite on the reduction of Fe³⁺ ions and transport processes within the passive film. *Electrochimica Acta*, 127, 7–19. <https://doi.org/10.1016/j.electacta.2014.01.165>.
- Qin, W., Jiao, F., Sun, W., He, M., & Huang, H. (2012). Selective flotation of chalcopyrite and marmatite by MBT and electrochemical analysis. *Industrial and Engineering Chemistry Research*, 51(35), 11538–11546. <https://doi.org/10.1021/ie300410f>.
- Saavedra, A., García-Meza, J. V., Cortón, E., & González, I. (2018). Understanding galvanic interactions between chalcopyrite and magnetite in acid medium to improve copper (Bio)Leaching. *Electrochimica Acta*, 265, 569–576. <https://doi.org/10.1016/j.electacta.2018.01.169>.
- Sandström, A., Shchukarev, A., & Paul, J. (2005). XPS characterisation of chalcopyrite chemically and bio-leached at high and low redox potential. *Minerals Engineering*, 18(5), 505–515. <https://doi.org/10.1016/j.mineng.2004.08.004>.
- Shirley, D. A. (1972). High-resolution x-ray photoemission spectrum of the valence bands of gold. *Physical Review B*, 5(12), 4709–4714. <https://doi.org/10.1103/PhysRevB.5.4709>.
- Song, C., Wen-qing, Q., & Guan-zhou, Q. (2008). Effect of Cu²⁺ ions on bioleaching of marmatite. *Transactions of Nonferrous Metals Society of China*, 1518–1522. [https://doi.org/10.1016/s1003-6326\(09\)60035-2](https://doi.org/10.1016/s1003-6326(09)60035-2).
- Vilcáez, J., Yamada, R., & Inoue, C. (2009). Effect of pH reduction and ferric ion addition on the leaching of chalcopyrite at thermophilic temperatures. *Hydrometallurgy*, 96(1–2), 62–71. <https://doi.org/10.1016/j.hydromet.2008.08.003>.
- Wang, Y., Chen, X., & Zhou, H. (2018). Relationships between galvanic interaction, copper extraction and community dynamics during bioleaching of chalcopyrite by a moderately thermophilic culture. *Bioresource Technology*, 265, 581–585. <https://doi.org/10.1016/j.biortech.2018.07.017>.
- Wang, X., Liao, R., Zhao, H., Hong, M., Huang, X., Peng, H., et al. (2018). Synergetic effect of pyrite on strengthening bornite bioleaching by *Leptospirillum ferriphilum*. *Hydrometallurgy*, 176, 9–16. <https://doi.org/10.1016/j.hydromet.2017.12.003>.

- Wen, X., Liang, Y., Bai, P., Luo, B., Fang, T., Yue, L., et al. (2017). First-principles calculations of the structural, elastic and thermodynamic properties of mackinawite (FeS) and pyrite (FeS₂). *Physica B: Condensed Matter*, 525, 119–126. <https://doi.org/10.1016/j.physb.2017.09.007>.
- Xiao, Y., Liu, X., Dong, W., Liang, Y., Niu, J., Gu, Y., et al. (2017). Effects of pyrite and sphalerite on population compositions, dynamics and copper extraction efficiency in chalcopyrite bioleaching process. *Archives of Microbiology*, 199(5), 757–766. <https://doi.org/10.1007/s00203-017-1342-9>.
- Yang, Y., Harmer, S., & Chen, M. (2015). Synchrotron-based XPS and NEXAFS study of surface chemical species during electrochemical oxidation of chalcopyrite. *Hydrometallurgy*, 156, 89–98. <https://doi.org/10.1016/j.hydromet.2015.05.011>.
- Yi, Y., Weihua, L., Bhargava, S. K., Weimin, Z., & Miao, C. (2016). A XANES and XRD study of chalcopyrite bioleaching with pyrite. *Minerals Engineering*, 157–162. <https://doi.org/10.1016/j.mineng.2016.01.019>.
- Zhang, Q., Xu, Z., Bozkurt, V., & Finch, J. A. (1997). Pyrite flotation in the presence of metal ions and sphalerite. *International Journal of Mineral Processing*, 52(2–3), 187–201. [https://doi.org/10.1016/s0301-7516\(97\)00064-1](https://doi.org/10.1016/s0301-7516(97)00064-1).
- Zhao, H., Wang, J., Gan, X., Zheng, X., Tao, L., Hu, M., et al. (2015). Effects of pyrite and bornite on bioleaching of two different types of chalcopyrite in the presence of *Leptospirillum ferriphilum*. *Bioresource Technology*, 194, 28–35. <https://doi.org/10.1016/j.biortech.2015.07.003>.
- Zhao, H., Wang, J., Hu, M., Qin, W., Zhang, Y., & Qiu, G. (2013). Synergistic bioleaching of chalcopyrite and bornite in the presence of *Acidithiobacillus ferrooxidans*. *Bioresource Technology*, 149, 71–76. <https://doi.org/10.1016/j.biortech.2013.09.035>.
- Zhao, H., Wang, J., Yang, C., Hu, M., Gan, X., Tao, L., et al. (2015). Effect of redox potential on bioleaching of chalcopyrite by moderately thermophilic bacteria: An emphasis on solution compositions. *Hydrometallurgy*, 151, 141–150. <https://doi.org/10.1016/j.hydromet.2014.11.009>.
- Zhao, H., Zhang, Y., Zhang, X., Qian, L., Sun, M., Yang, Y., et al. (2019). The dissolution and passivation mechanism of chalcopyrite in bioleaching: An overview. *Minerals Engineering*, 136, 140–154. <https://doi.org/10.1016/j.mineng.2019.03.014>.

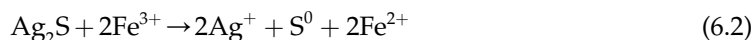
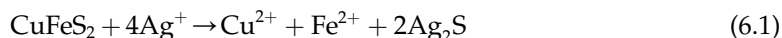
Chalcopyrite bioleaching catalyzed by silver

6.1 Catalytic mechanism of silver ions in chalcopyrite bioleaching

Silver ions (Ag^+) have been widely used as important and versatile catalyst for a variety of organic synthesis reactions (Álvarez-Corral et al., 2008; Naodovic & Yamamoto, 2008; Weibel et al., 2008). They can also be used as catalyst in inorganic reactions. For example, Ag^+ has been reported to have remarkable catalysis in the biohydrometallurgical process of sulfide minerals and secondary resources, especially in copper biohydrometallurgy (Ahonen & Tuovinen, 1990; Chen & Lin, 2009; Deng & Liao, 2002; Gómez et al., 1999; Guo et al., 2011; Nazari et al., 2012; Tapera et al., 2018). Ag^+ has been found to be an effective catalyst in chalcopyrite biohydrometallurgy, and the dissolution kinetics of chalcopyrite in the presence of Ag^+ can be remarkably accelerated (Ahonen & Tuovinen, 1990; Gómez et al., 1999; Hiroyoshi et al., 2002; Nazari et al., 2012).

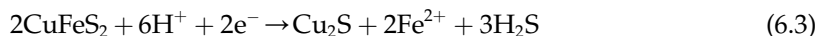
Many studies have suggested that Ag^+ catalyzed the oxidative dissolution process of chalcopyrite in bioleaching. Abdollahi et al. (Abdollahi et al., 2014, 2015) successively used Ag_2SO_4 and AgNO_3 as catalysts to bioleach a chalcopyrite-containing molybdenite concentrate; both high dissolution kinetics were obtained. Sato et al. (Sato et al., 2000) proposed that chloride (AgCl) had a better effect than silver sulfate at the early stage of chalcopyrite bioleaching. In addition, some studies found that silver-containing ores or waste also catalyzed the dissolution process of chalcopyrite in bioleaching (Yuehua et al., 2002).

The silver catalyzed mechanism in chalcopyrite bioleaching has been widely studied. Miller et al. (Miller et al., 1981) suggested that the formed elemental sulfur could be transformed into a porous state under the action of Ag^+ , and a direct catalysis mechanism model was further proposed (Eqs. 6.1 and 6.2): Subsequently, several studies have also supported the above model (Sato et al., 2000; Wang et al., 2004).



Price and Warren (Price & Warren, 1986) found that Ag^+ formed Ag_2S on a chalcopyrite surface, which enhanced conductivity and changed the morphology of the passivation layer. This result was supported by another study that further proposed that the formation of Ag_2S was caused by the reaction between Ag^+ and elemental sulfur (Nazari et al., 2012). Muñoz et al. (Muñoz et al., 1998) claimed that Ag_2S film and metallic silver both appeared on the surface of chalcopyrite.

In contrast, an indirect mechanism model was Eqs. (6.3) and (6.4). This model suggested that chalcopyrite was initially degraded to Cu_2S , which could be rapidly dissolved. The reaction between Ag^+ and H_2S speeded the reaction of Eq. (6.3), thus expediting chalcopyrite dissolution (Hiroyoshi et al., 2002):



Another model was proposed to illustrate the electrochemical dissolution of chalcopyrite catalyzed by Ag^+ . In this model, the formation of each Ag_2S molecule creates a sulfur vacancy and a pair of holes in the passivation layer; then, the porous passivation layer appears, accelerating the dissolution of chalcopyrite (Ghahremaninezhad et al., 2015).

Despite the remarkable catalytic effect of silver on chalcopyrite bioleaching, the high price of silver and challenges of effectively recovering silver from leached residues limit its industrial application. It is vital to illuminate the detailed forming process of silver-containing intermediate species and its role in the catalytic process.

6.1.1 Dissolution experiments

Chalcopyrite samples of high purity were obtained from the geological museum of the Guangxi Province of China and were carefully selected. Mineralogical analysis proved that they were of extremely high purity. Ore samples were ground by a ceramic ball mill and then sieved through a 200-mesh sieve. Finally, all of the undersize products (-0.074 mm) were used for the dissolution experiments. In the dissolution experiments, 2 g of chalcopyrite and 100 mL of diluted sulfuric acid solution were added to separate 250-mL shake flasks, which were placed in an orbital shaker at 45°C and a rotating speed of 170 rpm. An Ag_2SO_4 solution containing 1000 parts per million (ppm) Ag^{2+} was prepared in advance using deionized water, and then the 2 ppm Ag^{2+} was obtained by adding 0.2 mL of the Ag_2SO_4 solution to the corresponding flasks. *Leptospirillum ferriphilum* (CCTC AB 206239) was obtained from the Key Lab of Biohydrometallurgy of Ministry of Education, Central South University, Changsha, China. During the whole dissolution process, the pH was maintained at around 1.70 with sulfuric acid, and the evaporative water was supplemented regularly with deionized water. Cupric and total iron concentrations were analyzed periodically by inductively coupled plasma–optical emission spectrometry (ICAP 7400, Thermo Fisher Scientific Co.). The redox potentials and pH values of solution were detected by an oxidation-reduction potential (ORP) meter (BPP-922) and pH meter (PHSJ-4A).

Fe^{3+} is the main oxidant in the sulfuric acid-ferric sulfate system of chalcopyrite, and the redox potential, which is mainly determined by the $\text{Fe}^{3+}/\text{Fe}^{2+}$ ratio, can reflect the oxidative atmosphere of the chosen system. The dissolution process of chalcopyrite in a sulfuric acid-ferric sulfate system significantly depends on the redox potential (Córdoba et al., 2008; Hiroyoshi et al., 2008; Petersen & Dixon, 2006; Sandström et al., 2005). Hence, experiments of chalcopyrite dissolution by different $\text{Fe}^{3+}/\text{Fe}^{2+}$ ratios in the presence or absence of Ag^+ were conducted to investigate the catalytic mechanism of silver in the dissolution process of chalcopyrite.

Fig. 6.1A shows that the dissolution kinetics of Cu was extremely slow without the $\text{Fe}^{3+}/\text{Fe}^{2+}$ redox couple, and no significant catalytic effect of Ag^+ can be found under this condition.

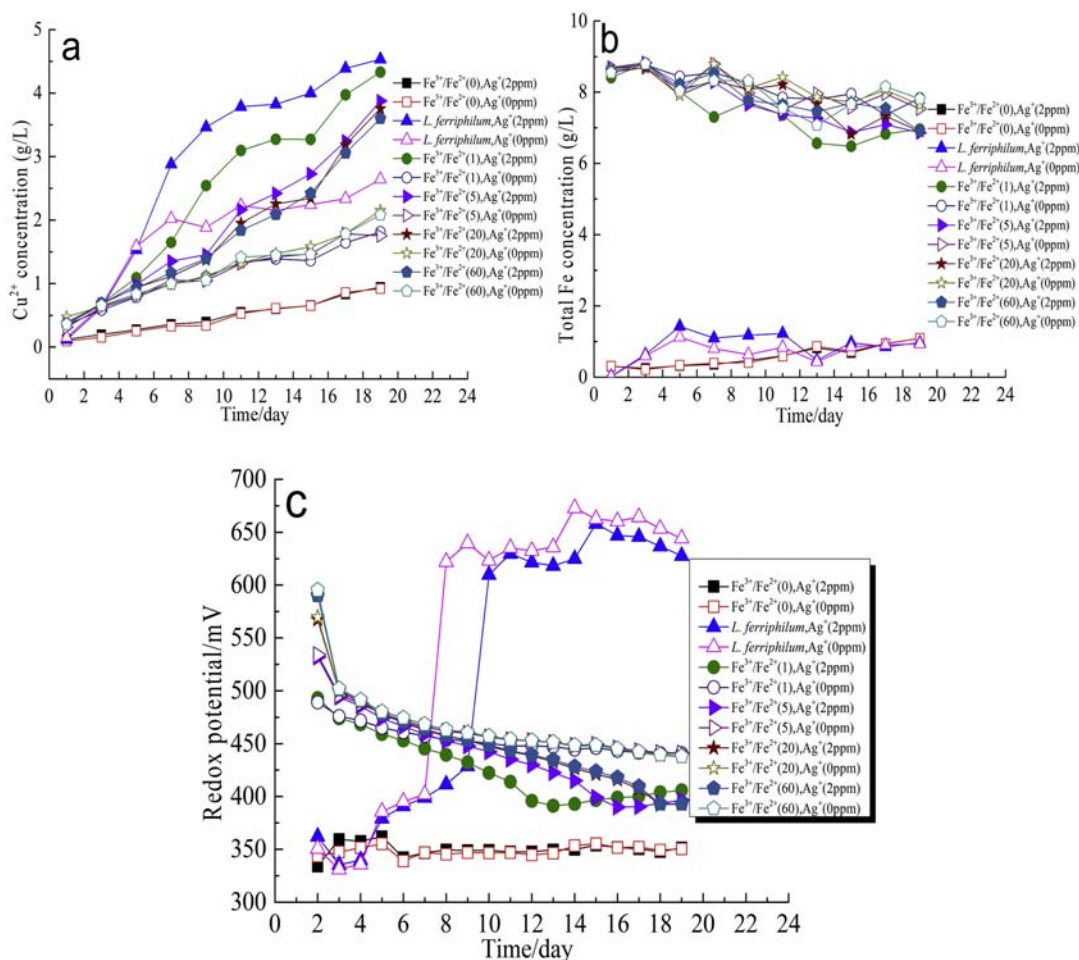


FIGURE 6.1 Dissolution behaviors of chalcopyrite under different conditions: (A) Cu^{2+} concentration; (B) Total Fe concentration; (C) Redox potential. ppm, parts per million. From Zhao, H., Zhang, Y., Sun, M., Ou, P., Zhang, Y., Liao, R., & Qiu, G. (2019). Catalytic mechanism of silver in the oxidative dissolution process of chalcopyrite: Experiment and DFT calculation. *Hydrometallurgy*, 187, 18–29. <https://doi.org/10.1016/j.hydromet.2019.05.002>.

The dissolution kinetic of Cu from chalcopyrite can be enhanced by adding the $\text{Fe}^{3+}/\text{Fe}^{2+}$ redox couple. An obvious catalytic effect of Ag^+ occurred in the presence of the $\text{Fe}^{3+}/\text{Fe}^{2+}$ redox couple. Fig. 6.1B indicates that Ag^+ cannot accelerate the dissolution of Fe from chalcopyrite in a sulfuric acid-ferric sulfate system even by adding an $\text{Fe}^{3+}/\text{Fe}^{2+}$ redox couple. In addition, the dissolution behavior of chalcopyrite in a microbial system is similar to that of chalcopyrite in the presence of an $\text{Fe}^{3+}/\text{Fe}^{2+}$ redox couple because the iron-oxidizing microorganism of *L. ferriphilum* can continuously oxidize Fe(II) to Fe (III), providing an $\text{Fe}^{3+}/\text{Fe}^{2+}$ redox couple in the microbial system. Hence, Ag^+ can significantly catalyze the dissolution of Cu from chalcopyrite by adding an $\text{Fe}^{3+}/\text{Fe}^{2+}$ redox couple. However, the dissolution of Fe from chalcopyrite cannot be catalyzed by Ag^+ in a sulfuric acid-ferric sulfate system. The hydrometallurgical process of chalcopyrite depends on the redox potential because the oxidative dissolution of chalcopyrite contains a series of oxidation-reduction reactions. Fig. 6.1C shows that adding an $\text{Fe}^{3+}/\text{Fe}^{2+}$ redox couple remarkably increased the redox potential of the dissolution system. The redox potential of the dissolution system was mainly determined by the $\text{Fe}^{3+}/\text{Fe}^{2+}$ ratio and was almost not affected by Ag^+ . Hence, the catalytic effect of Ag^+ may not be attributed to control of the redox potential. Hiroyoshi et al. (Hiroyoshi et al., 2002) proposed that chalcopyrite was initially transformed to Cu_2S , which can be dissolved rapidly in an appropriate range of redox potential, resulting in high copper extraction. The reaction between Ag^+ and H_2S speeded the reaction of Cu_2S formation through Eqs. (6.3) and (6.4), expediting chalcopyrite dissolution. Ag^+ may catalyze chalcopyrite dissolution mainly by changing the structures or properties of the chalcopyrite surface.

The mineralogical compositions of solid samples dissolved under different conditions were analyzed by synchrotron radiation-based X-ray diffraction (SR-XRD) at beamline 4B9A at Beijing Synchrotron Radiation Facility in Beijing, China. The SR-XRD of chalcopyrite samples that dissolved for 20 days under different conditions is provided in Fig. 6.2. A large amount of jarosite and elemental sulfur was produced with the addition of the $\text{Fe}^{3+}/\text{Fe}^{2+}$ redox couple.

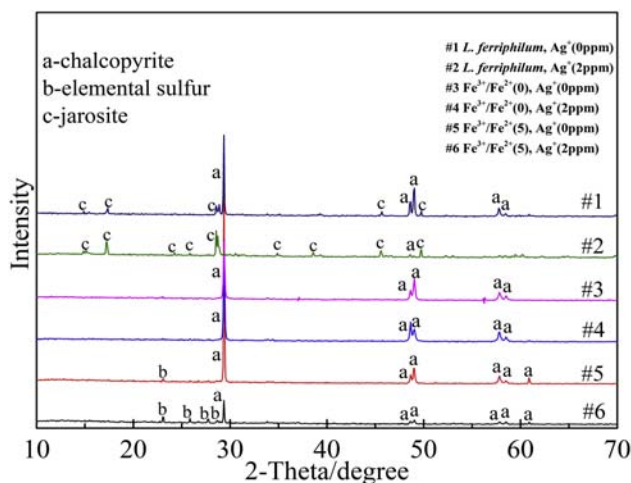


FIGURE 6.2 Synchrotron radiation-based X-ray diffraction of chalcopyrite samples dissolved for 20 days under different conditions. From Zhao, H., Zhang, Y., Sun, M., Ou, P., Zhang, Y., Liao, R., & Qiu, G. (2019). Catalytic mechanism of silver in the oxidative dissolution process of chalcopyrite: Experiment and DFT calculation. *Hydrometallurgy*, 187, 18–29. <https://doi.org/10.1016/j.hydromet.2019.05.002>.

The addition of Ag^+ even accelerated the production of jarosite and elemental sulfur. By contrast, the dissolution kinetics of chalcopyrite was slowest in the absence of the $\text{Fe}^{3+}/\text{Fe}^{2+}$ redox couple, but no significant amount of jarosite and elemental sulfur was detected in the dissolved chalcopyrite samples, which indicated that jarosite and elemental sulfur may not be the main passivating species in the sulfuric acid–ferric sulfate system of chalcopyrite. Although the passivation mechanism and specific passivating species in dissolution process of chalcopyrite are debated (Klauber, 2008), there was no doubt that the produced jarosite and elemental sulfur did not significantly hinder the dissolution of chalcopyrite in the presence of Ag^+ . In other words, the catalytic effect of Ag^+ overcame the adverse effects of the passivating species. Hence, it is likely that Ag^+ had changed the structures and properties of chalcopyrite surface, preventing passivation. To investigate the catalytic effects of Ag^+ on the structures and properties of the chalcopyrite surface, electrochemical tests, X-ray photoelectron spectroscopy (XPS) analysis and theoretical calculation were combined.

6.1.2 Density functional theory calculations on catalytic mechanism of Ag^+

Relaxation and total energy calculations based on density functional theory (DFT) (Hohenberg & Kohn, 1964; Kohn & Sham, 1965) were performed using the plane-wave based Vienna ab-initio simulation package (VASP) (Kresse & Furthmüller, 1996). The generalized-gradient approximation parametrized by Perdew–Burke–Ernzerhof (Perdew et al., 1996) was used to treat the exchange-correlation effects. Interactions between atomic cores and electrons were represented through the projector augmented-wave method of Blöchl (Blöchl, 1994; Kresse & Joubert, 1999) as implemented in VASP. The optimized cutoff energy of 400 eV was adopted throughout, which was sufficient for optimizing the slab models. In geometry optimizations, the total energy was converged to 10^{-5} eV, and forces acting on ions were required to be less than 0.02 eV/Å. Spin-polarization correction was addressed in our calculations.

To simulate the sulfur-terminated CuFeS_2 (001) and (112) surfaces (labeled as [001]-S and [112]-S), we used supercell models containing 2×2 (001)-S and 2×1 (112)-S slabs with eight atomic layers, which were sufficient for considering calculated surface and adsorption properties. A vacuum layer of 15 Å along the vertical direction perpendicular to the surface was employed to avoid spurious interactions between two adjacent slabs. In calculations of adsorbate adsorption and diffusion, the four uppermost surface layers were allowed to relax fully, whereas the remaining layers were fixed at the optimized bulk positions. The atomic positions were relaxed using a conjugate-gradient algorithm during the geometry optimization. The Brillouin zone integrations were performed using the Monkhorst-Pack scheme (Monkhorst Hendrik & Pack James, 1976) with $3 \times 3 \times 1$ k -point grids for both the (001)-S and (112)-S surfaces, applying a generalized Gaussian smearing method with the smearing width set to 0.1 eV.

To determine the exact reaction pathways for the catalytic mechanism of Ag^+ in chalcopyrite dissolution, first the adsorption of Ag^+ ions on the reconstructed (001)-S and (112)-S surfaces of chalcopyrite was studied by DFT calculations. In the following discussion, the high symmetry positions in the topmost two layers exposed to the surface were chosen as the potential adsorption active sites. Several typical configurations were optimized by considering

different initial orientations of adsorbate, in which the Ag^+ adsorbs on the top of Cu, Fe, or S atoms, in the hollow sites, and above the centers of Cu–S and Fe–S bonds (as shown in Fig. 6.3). Generally, the adsorption energy is considered a measure of the strength of substrate–adsorbate interaction. In the following discussion, adsorption energy E_{ad} calculated in this work is defined as Eq. (6.5):

$$E_{\text{ads}} = E(\text{slab} + \text{adsorbate}) - E(\text{slab}) - E(\text{adsorbate}) \quad (6.5)$$

in which the $E(\text{Slab} + \text{adsorbate})$ is the calculated total energy of surface with adsorbed Ag^+ on it, and (Slab) and (adsorbate) are the calculated energies of clean surface and isolated Ag^+ ion, respectively. Accordingly, a negative E_{ad} value corresponds to a stable interaction system in which the more negative the energy is, the stronger the adsorption system is.

For the Ag^+ ion adsorption, the optimized configurations are shown in Fig. 6.4, and the corresponding initial and final configurations and adsorption energies for all possible adsorption sites are summarized in Table 6.1. In the case of the (001)-S surface (cf. Fig. 6.4A and B), the Ag^+ ion is coordinated in the Cu_2Fe_2 hollow site and breaks the S–S dimer (site 2 in Fig. 6.3B) with the Ag–S bond length of 2.63 Å, and S–Ag–S forms an angle of 176.9 degrees. The adsorption energy was calculated to be -2.83 eV, which indicates that the adsorption of Ag^+ ion on the (001)-S surface is significantly energetically favorable. Moreover, when the

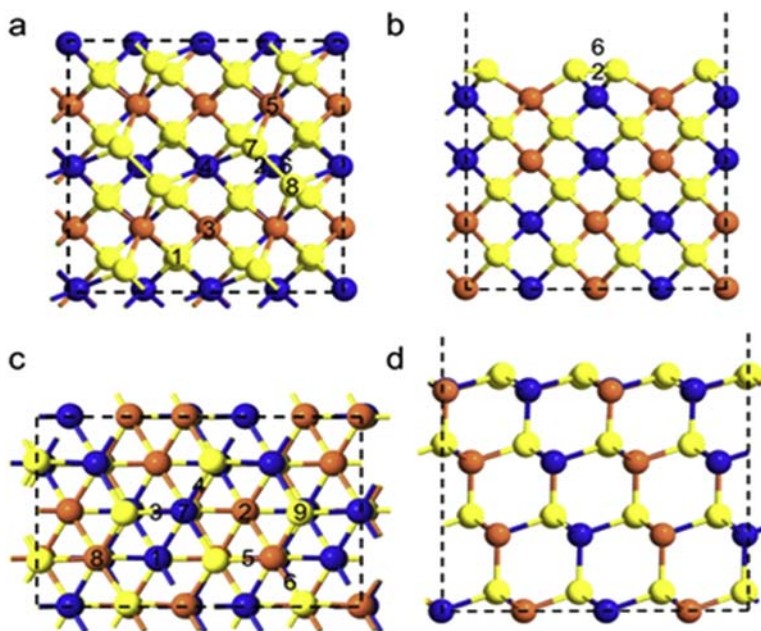


FIGURE 6.3 Various adsorption sites considered in the current study when determining the most stable configuration of the first Ag^+ ion adsorbed on the reconstructed. (A, B) (001)-S and (C, D) (112)-S surfaces. For the (001)-S surface, the 2 and 6 adsorption sites are overlapped in the top view. However, in the side view, 6 is at the top of an S–S bond, whereas 2 is in the hollow site made of two copper atoms and two iron atoms. The orange, blue, and yellow balls represent iron, copper, and sulfur atoms, respectively. From Zhao, H., Zhang, Y., Sun, M., Ou, P., Zhang, Y., Liao, R., & Qiu, G. (2019). Catalytic mechanism of silver in the oxidative dissolution process of chalcopyrite: Experiment and DFT calculation. *Hydrometallurgy*, 187, 18–29. <https://doi.org/10.1016/j.hydromet.2019.05.002>.

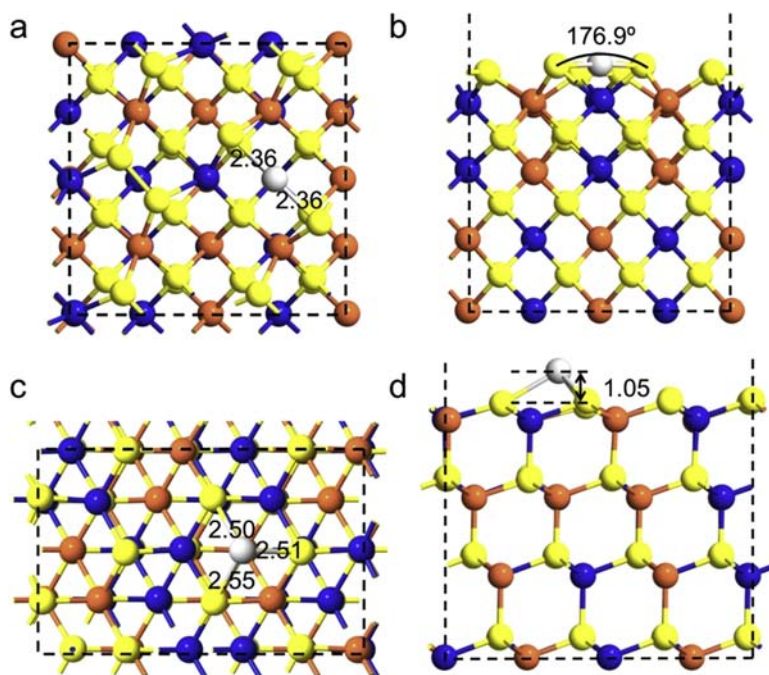


FIGURE 6.4 The most stable adsorption site of the first Ag^+ ion adsorbed on the reconstructed (A, B) (001)-S and (C, D) (112)-S surfaces. The orange, blue, yellow, and light gray balls represent iron, copper, sulfur, and silver atoms, respectively. From Zhao, H., Zhang, Y., Sun, M., Ou, P., Zhang, Y., Liao, R., & Qiu, G. (2019). Catalytic mechanism of silver in the oxidative dissolution process of chalcopyrite: Experiment and DFT calculation. *Hydrometallurgy*, 187, 18–29. <https://doi.org/10.1016/j.hydromet.2019.05.002>.

Ag^+ ion is adsorbed on the S_Top1 site (site 7 in Fig. 6.3A), it will automatically move to the Cu_2Fe_2 hollow site and the adsorption energy will be similar to that of Cu_2Fe_2 hollow site. In terms of the (112)-S surface, as shown in Fig. 6.4C and D, the Ag^+ ion tends to be adsorbed in the Cu_2Fe_1 hollow site (site 2 in Fig. 6.3C), with a smaller negative adsorption energy of -0.66 eV compared with that of the (001)-S surface, residing 1.05 Å higher than the top S plane. The Ag^+ ion connects with three neighboring S atoms with the bond lengths of 2.50 , 2.51 , and 2.55 Å, respectively. Considering these results, we believe that the hollow site of chalcopyrite surface shows a significant adsorption interaction with the Ag^+ ion.

Moreover, to gain insights into the Ag^+ ion catalysis of chalcopyrite dissolution at a molecular level, DFT calculations were performed to understand the reaction energetics. As suggested in a previous study (Ghahremaninezhad et al., 2015), the catalytic mechanism of Ag^+ in chalcopyrite dissolution was considered through the following steps:

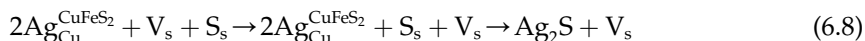
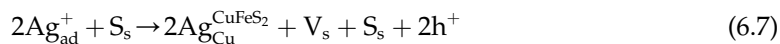


TABLE 6.1 Initial and optimized final configurations (corresponding to adsorption sites in Fig. 6.3) together with density functional theory calculated adsorption energies (E_{ad} , in eV) of first Ag^+ ion adsorbed on reconstructed (001)-S and (112)-S surfaces.

Surface	No.	Initial	Final	E_{ad}
(001)-S	1	Cu ₂ Fe ₂ _Hollow1	—	−1.83
	2	Cu₂Fe₂_Hollow2	—	−2.83
	3	CuAgFe_Bridge	Moved to Cu ₂ Fe ₂ _Hollow1	−1.83
	4	S_Cu_S_Bridge	—	−1.69
	5	S_Fe_S_Bridge	—	−2.02
	6	S_S_Bridge	—	0.52
	7	S_Top1	Moved to Cu ₂ Fe ₂ _Hollow2	−2.79
	8	S_Top2	Moved to Cu ₂ Fe ₂ _Hollow1	−1.83
(112)-S	1	Cu ₁ Fe ₂ _Hollow	Formed an Ag–Fe bond	−0.47
	2	Cu₂Fe₁_Hollow	—	−0.66
	3	S_Cu_Bridge1	Moved to Cu_Top	−0.26
	4	S_Cu_Bridge2	Moved to Cu_Top	−0.05
	5	S_Fe_Bridge1	Partially moved to Cu ₂ Fe ₁ _Hollow	−0.25
	6	S_Fe_Bridge2	Moved to Cu ₁ Fe ₂ _Hollow	0.32
	7	Cu_Top	—	−0.05
	8	Fe_Top	—	−0.39
	9	S_Top	Partially moved to Cu ₂ Fe ₁ _Hollow	−0.25

Bolded values indicate energetically favorable adsorption configuration. Dashes indicate that final configuration of Ag^+ ion adsorption remained the same as initial configuration.

where Ag^+_{aq} and Ag^+_{ad} denote a silver ion in the solution and adsorbed on the surface of the chalcopyrite passive film, $AgCu^{CuFeS}_2$ indicates a silver atom is adsorbed in a copper (or iron) vacancy in the chalcopyrite passive film, V_S is for a sulfur vacancy, S_S denotes a sulfur atom in a sulfur position in the $CuFeS_2$ structure, and h^+ refers the positive charge carriers (i.e., holes). Eq. (6.6) describes the adsorption of Ag^+ ions onto the surface of the chalcopyrite passive film, and Eq. (6.7) expresses an electrochemical reaction of Ag_2S formation. The formation of Ag_2S molecule in Eq. (6.8) introduces a sulfur vacancy to the chalcopyrite film; subsequently, the silver atoms and sulfur vacancies might diffuse into the passive film structure.

According to Eqs. (6.6)–(6.8), the DFT calculated results for four different steps in the reaction mechanism on (001)-S and (112)-S surfaces are shown in Fig. 6.5A and B, respectively, denoted as first Ag^+ adsorption and second Ag^+ adsorption (along with the formation of an S vacancy on the chalcopyrite surface), Ag_2S formation, and Ag_2S diffusion (equivalent to the diffusion of silver into the chalcopyrite passive film). On the (001)-S surface, the first step of first Ag^+ ion adsorption on the surface is highly exothermic, with an energy change

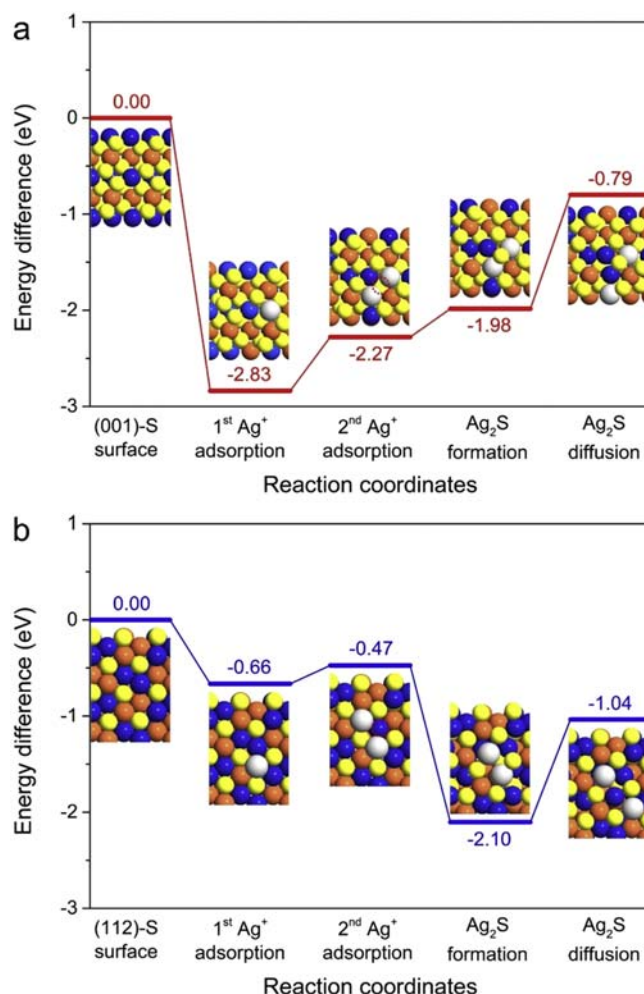


FIGURE 6.5 Density functional theory calculated energy difference and optimized configurations for catalytic mechanism of Ag^+ in chalcopyrite dissolution on reconstructed (A) (001)-S and (B) (112)-S surfaces. Four steps are shown: First Ag^+ adsorption, second Ag^+ adsorption (along with the formation of an S vacancy on the chalcopyrite surface), Ag_2S formation, and Ag_2S diffusion (equivalent to the diffusion of silver into the chalcopyrite passive film). Orange, blue, yellow, and light gray spheres represent iron, copper, sulfur, and silver atoms, respectively. The sulfur vacancy is shown as a dashed red circle (seen in the 2nd Ag^+ adsorption patterns in Fig. 6.5 A and B). From Zhao, H., Zhang, Y., Sun, M., Ou, P., Zhang, Y., Liao, R., & Qiu, G. (2019). Catalytic mechanism of silver in the oxidative dissolution process of chalcopyrite: Experiment and DFT calculation. *Hydrometallurgy*, 187, 18–29. <https://doi.org/10.1016/j.hydromet.2019.05.002>.

of -2.83 eV. The following reactions are consecutively endothermic reactions: 0.56 eV for second Ag^+ adsorption, 0.29 eV for Ag_2S formation, and 1.19 eV, which is the rate-limiting step for Ag_2S diffusion, respectively. In contrast, on the (112)-S surface, the first Ag^+ ion adsorption is less energetically favorable, with an energy release of -0.66 eV compared with that of the (001)-S surface. After the adsorption of the first Ag^+ ion, the second Ag^+ ion adsorption is an endothermic reaction with energy slightly increased by 0.19 eV, followed by an

exothermic reaction of Ag_2S formation with a large energy drop of -1.63 eV. The last step of Ag_2S diffusion with the diffusion of an Ag^+ ion into the chalcopyrite film is highly endergonic, with an energy change of 1.06 eV, which is the rate-limiting step for the whole catalytic mechanism on the (112)-S surface. From the calculation results, the energy diagrams for the Ag^+ ion catalysis of chalcopyrite dissolution on the (001)-S and (112)-S surfaces are different, but the last step of Ag^+ ion diffusion is the same rate-limiting step with similar energy barriers of 1.19 and 1.06 eV, respectively.

6.1.3 Surface species

To investigate the dissolution process of chalcopyrite under different conditions, the types and distributions of surface species of chalcopyrite electrodes treated under different conditions were analyzed using XPS. XPS analysis was conducted using the model ESCALAB 250Xi. Spectra were recorded at a constant pass energy of 20 and 0.1 eV/step using an Al K_α X-ray source.

Fig. 6.6 shows that Cu-, Fe-, S-, and O-containing species were the main surface species of chalcopyrite treated by air or H_2SO_4 without Ag^+ . However, Ag-containing species can be detected on the chalcopyrite surface treated by H_2SO_4 with Ag^+ . The surface atomic percentages obtained by XPS surface survey analyses are shown in Table 6.2.

To investigate further the specific species of chalcopyrite surface under different conditions, the XPS spectra of Ag 3d, Cu 2p, Fe 2p, and Fe 3p were analyzed (Fig. 6.7). The obtained XPS spectra were fitted using Thermo Avantage 5.52 software. During the fitting process, the binding energies of spectra were referred to the C 1s level at 284.8 eV. The background was achieved using the Shirley method, and the spectra were fitted by the Gaussian–Lorentzian line function (Shirley, 1972). Fig. 6.7A shows that the Ag $3d_{5/2}$ peak of chalcopyrite treated by Ag^+ was centered at 367.8 eV, which was similar to the binding energy of Ag–S species (Palacio et al., 2003). However, no significant Ag 3d peak could be detected in the other two

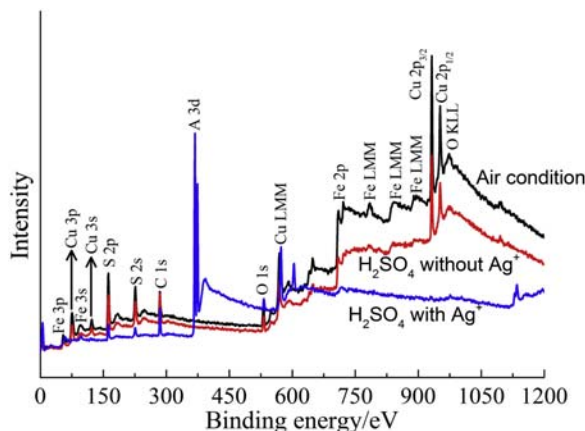


FIGURE 6.6 Survey (full-range) X-ray photoelectron spectroscopy spectra of chalcopyrite treated under different conditions. KLL, KLL auger peak; LMM, LMM auger peak. From Zhao, H., Zhang, Y., Sun, M., Ou, P., Zhang, Y., Liao, R., & Qiu, G. (2019). Catalytic mechanism of silver in the oxidative dissolution process of chalcopyrite: Experiment and DFT calculation. *Hydrometallurgy*, 187, 18–29. <https://doi.org/10.1016/j.hydromet.2019.05.002>.

TABLE 6.2 Surface atomic percentages.

Surface atomic percentages (%)	Cu	Fe	S	Ag	O
H ₂ SO ₄ + Ag ⁺	2.38	1.21	31.72	49.58	15.11
H ₂ SO ₄	26.97	11.31	47.48	—	14.24
Air	29.48	14.09	43.02	—	13.41

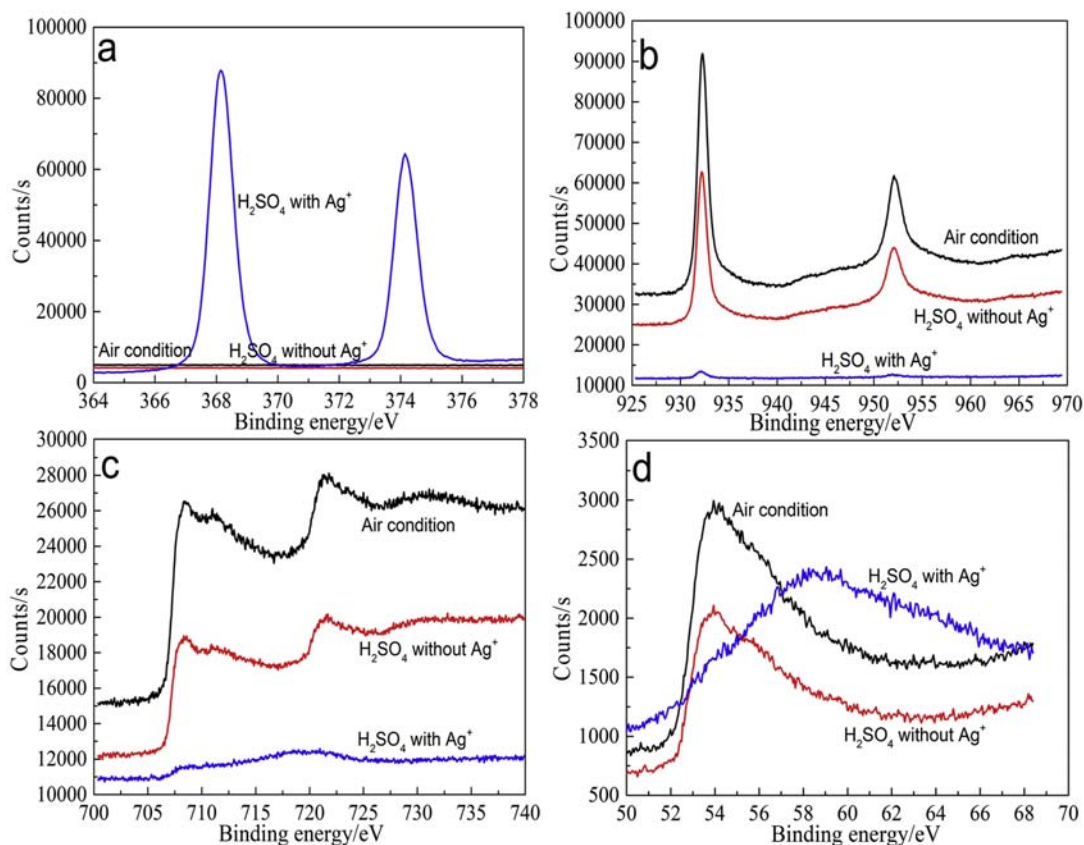


FIGURE 6.7 X-ray photoelectron spectroscopy spectra of Ag 3d (A), Cu 2p (B), Fe 2p (C), and Fe 3p (D) of chalcopyrite treated under different conditions. From Zhao, H., Zhang, Y., Sun, M., Ou, P., Zhang, Y., Liao, R., & Qiu, G. (2019). Catalytic mechanism of silver in the oxidative dissolution process of chalcopyrite: Experiment and DFT calculation. *Hydrometallurgy*, 187, 18–29. <https://doi.org/10.1016/j.hydromet.2019.05.002>.

chalcopyrite samples treated without Ag⁺. Cu 2p_{3/2} peaks of chalcopyrite samples treated with no Ag⁺ were both centered at about 932.0 eV, in accordance with the binding energy of Cu–S species of chalcopyrite, Cu₂S, or CuS (Fujisawa et al., 1994; Nakai et al., 1978). However, the Cu 2p peaks of chalcopyrite treated by Ag⁺ were remarkably weakened and almost disappeared. This may have been caused by the high diffusion rate of copper atoms of the

chalcopyrite surface and by the coverage of the formed silver sulfide. The XPS spectra of Fe, especially Fe 3p peaks, significantly shifted because of the treatment of Ag^+ , which indicated that the surrounding chemical environment of Fe atoms in the lattice structure of the chalcopyrite surface had changed because of the chemical reactions between Ag^+ and chalcopyrite. The obtained results further supported the results of theoretical calculations.

The S 2p spectra of chalcopyrite samples treated under different conditions (Fig. 6.8) were fitted using a 2:1 peak area ratio and 1.2 eV splitting for S 2p_{3/2} and S 2p_{1/2}. Monosulfide (S^{2-}), disulfide (S_2^{2-}), and polysulfide (S_n^{2-}) were the main sulfur-containing species of a chalcopyrite surface treated under different conditions. The percentages of sulfur-containing species on a chalcopyrite surface after treatment under different conditions were calculated from the peak area, shown in Fig. 6.8D. Polysulfide was previously reported to be the surface species of chalcopyrite formed through surface reconstruction under an air condition (Nasluzov et al., 2019). Some work proposed that a thin layer of polysulfide could be produced in the oxidative dissolution of chalcopyrite (Eq. 6.9). Poorly reactive and relatively dense metal-deficient polysulfide with a low conductivity and diffusion rate caused the passivation of

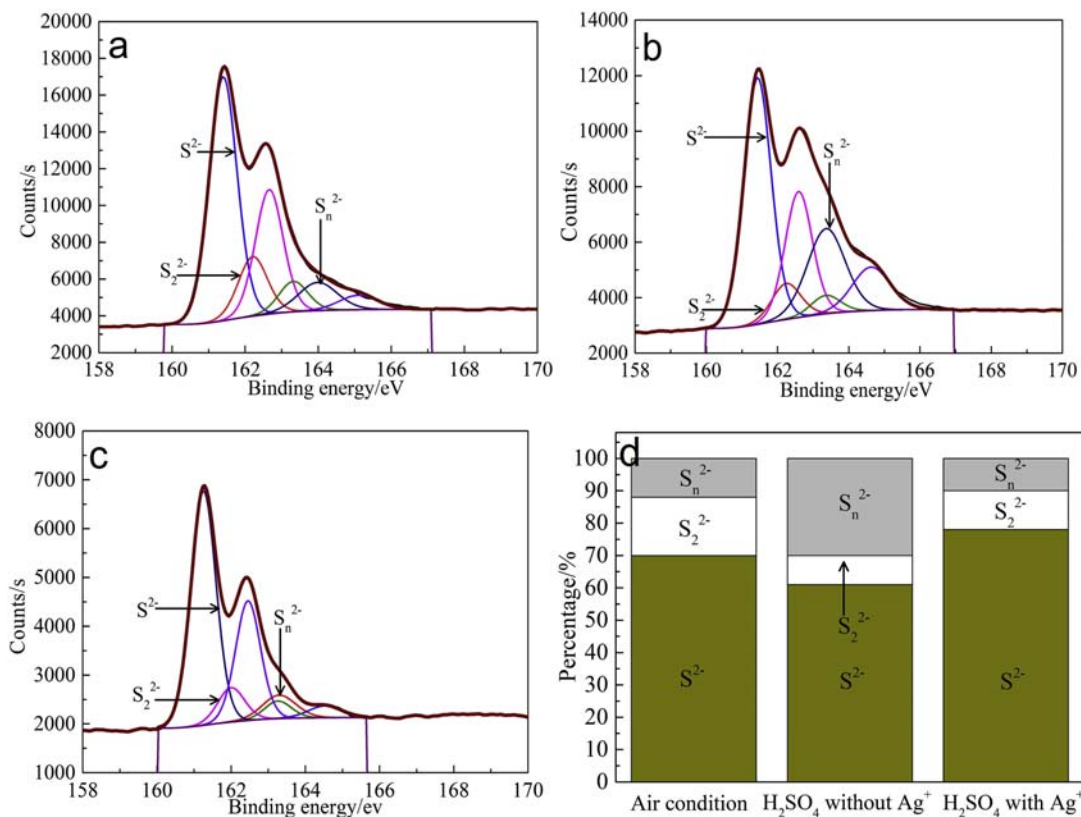
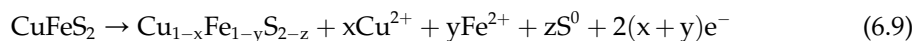


FIGURE 6.8 S 2p spectra of chalcopyrite treated under different conditions. (A) Air condition, (B) H_2SO_4 without Ag^+ , (C) H_2SO_4 with Ag^+ , (D) distribution of sulfur species on chalcopyrite surface. From Zhao, H., Zhang, Y., Sun, M., Ou, P., Zhang, Y., Liao, R., & Qiu, G. (2019). Catalytic mechanism of silver in the oxidative dissolution process of chalcopyrite: Experiment and DFT calculation. *Hydrometallurgy*, 187, 18–29. <https://doi.org/10.1016/j.hydromet.2019.05.002>.

chalcopyrite (Hackl et al., 1995; Linge, 1976; Mustapha et al., 1977; Parker et al., 1981; Yang et al., 2015). A large amount of polysulfide was produced on a chalcopyrite surface under air or H_2SO_4 . However, the percentage of polysulfide on the chalcopyrite surface greatly decreased when Ag^+ was added, which indicated that Ag^+ prevented the accumulation of passivating species of polysulfide and then intensified chalcopyrite dissolution. The formation and coverage of silver sulfide may cause a decrease in the percentage of polysulfide. To be exact, all surfaces detected by XPS cannot simply be chalcopyrite; it is better described using the term “reaction surface,” which is the real region of interfacial reactions.



The XPS spectra of O 1s peaks of chalcopyrite electrode treated under different conditions are provided in Fig. 6.9. The binding energy of O 1s at 529.6 ± 0.1 , 531.5 ± 0.1 , and 532.8 ± 0.1 eV are mainly associated with O^{2-} in oxide phases, OH^- in hydroxide species, and oxygen in sulfate (SO_4^{2-}) and/or water, respectively (Ghahremaninezhad et al., 2013). O^{2-} , OH^- , and

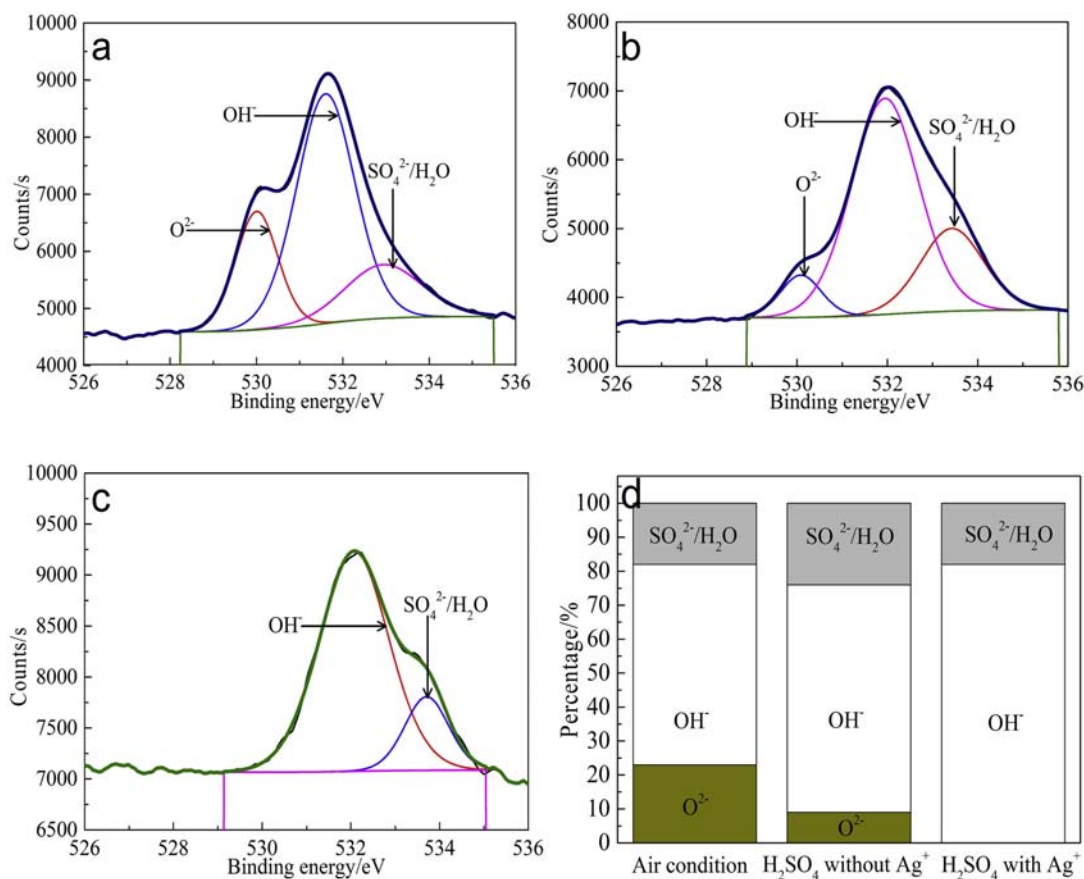
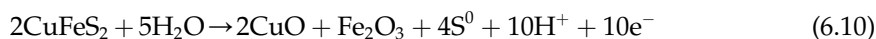


FIGURE 6.9 O 1s spectra of chalcopyrite treated under different conditions. (A) Air condition, (B) H_2SO_4 without Ag^+ , (C) H_2SO_4 with Ag^+ , (D) distribution of oxygen species on chalcopyrite surface. From Zhao, H., Zhang, Y., Sun, M., Ou, P., Zhang, Y., Liao, R., & Qiu, G. (2019). Catalytic mechanism of silver in the oxidative dissolution process of chalcopyrite: Experiment and DFT calculation. *Hydrometallurgy*, 187, 18–29. <https://doi.org/10.1016/j.hydromet.2019.05.002>.

SO_4^{2-} were the main oxygen-containing species on the chalcopyrite surface under the conditions of air or H_2SO_4 . Moreover, the percentage of hydroxide species on chalcopyrite in H_2SO_4 was higher than that in air, and the hydroxide species was mainly attributed to FeOOH . However, almost no significant amount of O^{2-} species could be detected on the chalcopyrite surface treated with Ag^+ . O^{2-} species was mainly assigned metallic oxides, which were also considered plausible passivating species in the dissolution process of chalcopyrite (similar to Eq. 6.10) (Ahmadi et al., 2010; Khoshkhoo et al., 2014; Li & Huang, 2011). Hence, Ag^+ may have prevented the accumulation of passivating species of metallic oxides.



6.1.4 Surface properties

Electrochemical experiments of open circuit potential (OCP), electrochemical impedance spectroscopy (EIS), Tafel, and cyclic voltammetry were conducted with a Princeton Model 283 potentiostat (EG&G, Princeton Applied Research). In the electrochemical experiments, a conventional three-electrode system consisted of a working electrode of chalcopyrite, a graphite rod as the counter electrode, and an Ag/AgCl (3.0 M KCl) electrode as the reference electrode. The electrochemical experiments were all conducted in an electrolyte of dilute sulfuric acid (pH 1.70) and an atmosphere of N_2 . The cyclic voltammetry test reveals the oxidation-reduction reactions of chalcopyrite at different applied potentials, so it can be used to interpret the thermodynamics and kinetics of the electrochemical dissolution process of chalcopyrite under different conditions. Fig. 6.10 presents the cyclic voltammetry curves of

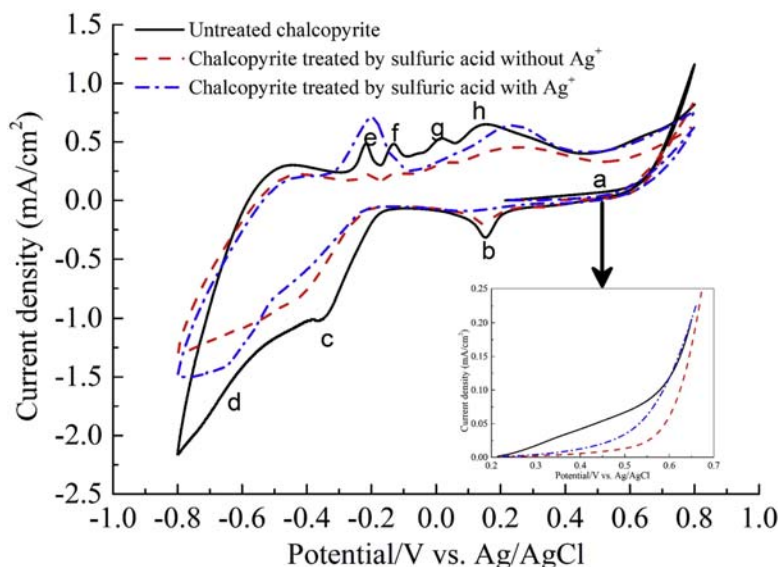


FIGURE 6.10 Cyclic voltammetry curves of chalcopyrite electrode treated under different conditions (20 mV/s). From Zhao, H., Zhang, Y., Sun, M., Ou, P., Zhang, Y., Liao, R., & Qiu, G. (2019). Catalytic mechanism of silver in the oxidative dissolution process of chalcopyrite: Experiment and DFT calculation. *Hydrometallurgy*, 187, 18–29. <https://doi.org/10.1016/j.hydromet.2019.05.002>.

chalcopyrite electrode treated under different conditions. The electrochemical dissolution processes of chalcopyrite under different conditions were similar. Adding Ag^+ did not obviously change the dissolution mechanism of chalcopyrite. The first anodic peak mainly represents the direct oxidation of chalcopyrite to metal-deficient polysulfide (S_n^{2-}) (Eq. 11). Many scholars proposed that the formed poorly reactive and relatively dense metal-deficient polysulfide with a low conductivity and diffusion rate caused the passivation of chalcopyrite (Hackl et al., 1995; Linge, 1976; Mustapha et al., 1977; Parker et al., 1981). It was difficult to oxidize the formed polysulfide directly by Fe^{3+} because of its relatively low oxidizing ability, so that stronger oxidants (such as potassium dichromate and nitric acid) were required to dissolve it effectively. The current density of peak a of chalcopyrite treated by sulfuric acid significantly decreased compared with that of untreated chalcopyrite, indicating that the formed polysulfide hindered the oxidative dissolution of chalcopyrite. However, the addition of Ag^+ significantly increased the current density of chalcopyrite, indicating that the addition of Ag^+ can accelerate the oxidative dissolution of chalcopyrite and reduce the passivation of polysulfide to a certain degree. The continuous anodic peaks of f, g, and h represent the oxidative dissolution of Cu_2S . The continuous peaks of g and h disappeared with the addition of Ag^+ . A possible explanation is that adding Ag^+ increased the electrochemical reactivity of chalcopyrite, leading to the direct oxidation of Cu_2S to CuS without the other intermediate reactions. Hence, adding Ag^+ did not significantly change the electrochemical dissolution mechanism of chalcopyrite but enhanced its electrochemical reactivity.

The value of OCP is a parameter reflecting the corrosion-resistant abilities of materials. A lower value of OCP indicates that materials can be more easily corroded or oxidized. On the other hand, a higher value of OCP means it is more difficult for materials to be oxidized. Fig. 6.11A shows that the OCP of chalcopyrite treated with sulfuric acid greatly increased compared with that of untreated chalcopyrite, indicating that it became more difficult to

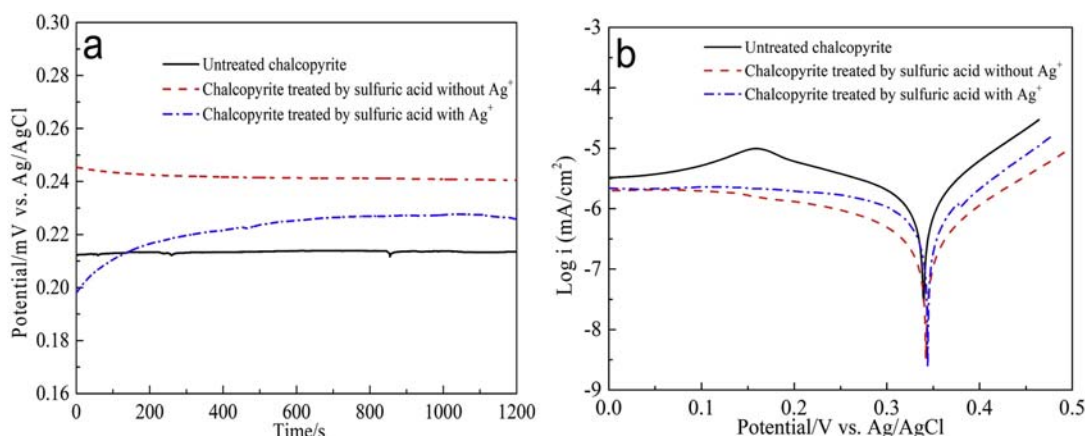


FIGURE 6.11 Open circuit potential (A) and Tafel curves (B) of chalcopyrite electrode treated under different conditions. From Zhao, H., Zhang, Y., Sun, M., Ou, P., Zhang, Y., Liao, R., & Qiu, G. (2019). Catalytic mechanism of silver in the oxidative dissolution process of chalcopyrite: Experiment and DFT calculation. *Hydrometallurgy*, 187, 18–29. <https://doi.org/10.1016/j.hydromet.2019.05.002>.

oxidize. The formed passivation layer may be responsible for the high OCP. However, adding Ag^+ significantly decreased the OCP, and adding Ag^+ reduced the adverse effect of the passivation layer.

The Tafel test can be used to analyze the corrosion kinetics of materials. Parameters of oxidative corrosion potential (E_{corr}) and corrosion current density (I_{corr}) can be used to analyze the corrosion kinetics (Fig. 6.11B). The corrosion current density of chalcopryite treated with sulfuric acid significantly decreased to about $0.478 \mu\text{A}/\text{cm}^2$ compared with $2.234 \mu\text{A}/\text{cm}^2$ for untreated chalcopryite, indicating that the oxidative corrosion kinetics of chalcopryite slowed because of passivation. However, the corrosion current density of chalcopryite treated with Ag^+ was about $1.066 \mu\text{A}/\text{cm}^2$, which was greatly increased compared with that of chalcopryite treated only with sulfuric acid ($0.478 \mu\text{A}/\text{cm}^2$). This shows that the oxidative corrosion kinetics of chalcopryite was enhanced by adding Ag^+ . Hence, adding Ag^+ reduced the adverse effect of the passivation layer by intensifying the oxidative corrosion kinetics.

EIS can be used to analyze the kinetics model of charge transfer. The EIS spectra were fitted and analyzed; the Nyquist impedance spectra and Bode plots are provided in Fig. 6.12 based on the proposed equivalent circuit. The well-fitted results of impedance parameters for the

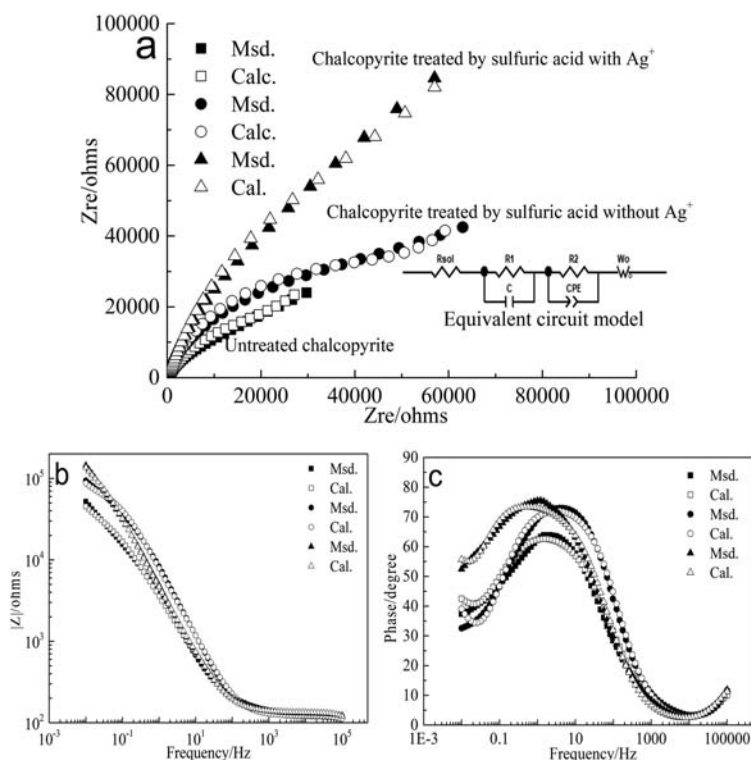


FIGURE 6.12 Electrochemical impedance spectroscopy of chalcopryite electrode treated under different conditions: (A) Nyquist impedance spectra, (B, C) Bode plots. Msd., Measured; Cal., Calculated. From Zhao, H., Zhang, Y., Sun, M., Ou, P., Zhang, Y., Liao, R., & Qiu, G. (2019). Catalytic mechanism of silver in the oxidative dissolution process of chalcopryite: Experiment and DFT calculation. *Hydrometallurgy*, 187, 18–29. <https://doi.org/10.1016/j.hydromet.2019.05.002>.

TABLE 6.3 Impedance parameters for equivalent circuit.

Element	R_{sol} (Ω/cm^2)	R_1 (Ω/cm^2)	C ($e-8 F/cm^2$)	R_2 (Ω/cm^2)	CPE ($Ss^{1/2}/cm^2$)		W_o ($Ss^{1/2}/cm^2$)		
					CPE-T ($e-5$)	CPE-P	W_o-R ($e-6$)	W_o-T ($e-12$)	W_o-P
Untreated	87.68	40.1	2.98	20,034	12.0	0.81	99.9	3.86	0.34
H ₂ SO ₄	69.59	52.22	1.67	54,195	3.04	0.86	7.61	5.97	0.40
H ₂ SO ₄ +Ag ⁺	83.77	49.79	2.17	43,672	10.5	0.96	12.2	2.04	0.39

CPE, Constant phase angle element; W_o , Warburg element (Open); CPE-T and CPE-P are the parameters of CPE; W_o-R , W_o-T and W_o-P are the parameters of W_o .

equivalent circuit are shown in Table 6.3. R_{sol} represents the solution resistance, R_1 means the charge transfer resistance, and R_2 represents the passivation layer resistance. The charge transfer resistance is shown in Eq. (6.11) (Ghahremaninezhad et al., 2012):

$$R_1 = \frac{RT}{nFi_0} \quad (6.11)$$

where parameters R , T , n , and F represent the ideal gas constant ($R = 8.314 J/K mol$), temperature (K), charge transfer number, and Faraday constant ($F = 96,494 C$), respectively. The value of i_0 represents the exchange current density of electrochemical reactions. A high value of i_0 and low value of R_1 indicate that the corresponding kinetics of electrochemical reactions was high, and vice versa. The charge transfer resistance of chalcopyrite treated with sulfuric acid greatly increased compared with that of untreated chalcopyrite, indicating that the electrochemical reactions were hindered. However, the charge transfer resistance of chalcopyrite significantly decreased because of the added Ag⁺. Similar results can be found in the parameters of passivation layer resistance (Table 6.3), shown in Eq. (6.12):

$$R_2 = \frac{R_1^2|B|}{a - R_1|B|} \quad (6.12)$$

where parameter a is the charge transfer coefficient. The passivation layer resistance of chalcopyrite treated with sulfuric acid greatly increased compared with that of untreated chalcopyrite, indicating that a passivation layer formed on the chalcopyrite surface and hindered the electrochemical reactions. The addition of Ag⁺ decreased the passivation layer resistance of chalcopyrite compared with that of chalcopyrite treated only with sulfuric acid, indicating that the presence of Ag⁺ reduced the adverse effect of the passivation layer by decreasing resistance and increasing the charge transfer rate.

6.1.5 Discussions on the catalytic mechanisms of Ag⁺

According to DFT calculations, the hollow site on the chalcopyrite surface shows a significant adsorption interaction with Ag⁺ ions and the formation of silver sulfide species, accompanied by the formation of sulfur vacancies. The sulfur vacancies formed in reaction (7) can be accountable for a high point defect concentration and result in high

electrochemical reactivity. In addition, sulfur vacancies are responsible for the formation of the chalcopyrite passive film covered with a porous surface layer, which was validated by experimental observations (Gómez et al., 1999; Muñoz et al., 2007; Sato et al., 2000; Wang et al., 2004; Yuehua et al., 2002). It was also confirmed that the formation of a porous surface layer resulted from both a sufficiently high rate of vacancy generation and the condensation of excess vacancies. Moreover, the diffusion of silver atoms (equivalent to Ag_2S diffusion in Fig. 6.5A and B) into the chalcopyrite passive film has a remarkable impact on chalcopyrite dissolution. The diffusion of silver atoms into the chalcopyrite passive film may induce significantly large lattice distortion owing to a larger ionic radius of Ag^+ (129 p.m.) compared with that of Cu^+ (91 p.m.). Therefore, we would expect the diffusion kinetics of copper (and iron) atoms to be accelerated in the silver-incorporated chalcopyrite passive film compared with the one without silver incorporation (Kadrgulov et al., 2001; Parker et al., 2003). XPS results of Cu 2p and Fe 3p (Fig. 6.7) and DFT calculations further supported these conclusions. On the other hand, combined with the analytical results of XPS and electrochemistry, it can be speculated that Ag^+ prevented the accumulation of passivating species of polysulfide and metallic oxides mainly by accelerating its further oxidative dissolution process (Eqs. 6.9 and 6.10). As a consequence, the presence of Ag^+ ions enhanced the electrochemical reactivity of chalcopyrite by creating silver sulfide species and sulfur vacancy, and then prevented the accumulation of passivating species, resulting in high dissolution kinetics. According to this work, a model for interpreting the catalytic mechanisms of Ag^+ in chalcopyrite dissolution is provided in Fig. 6.13.

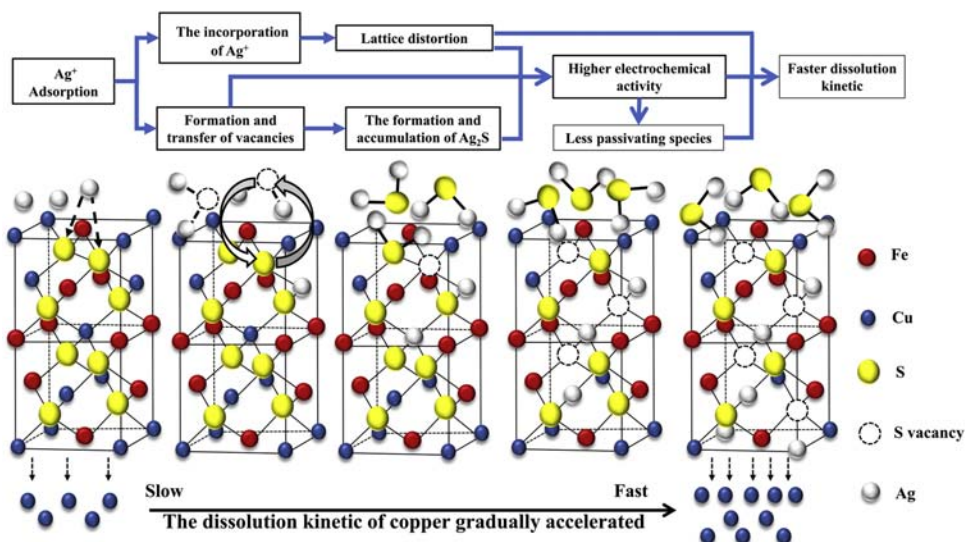


FIGURE 6.13 Model for interpreting catalytic mechanisms of Ag^+ in chalcopyrite dissolution. From Zhao, H., Zhang, Y., Sun, M., Ou, P., Zhang, Y., Liao, R., & Qiu, G. (2019). Catalytic mechanism of silver in the oxidative dissolution process of chalcopyrite: Experiment and DFT calculation. *Hydrometallurgy*, 187, 18–29. <https://doi.org/10.1016/j.hydromet.2019.05.002>.

6.2 Catalyzed bioleaching using silver-containing waste

Commercial application of silver-catalyzed technology is limited owing to the high price of silver and difficulties of recovering silver used for catalysis. More than 80% of the total metal zinc is produced through the conventional hydrometallurgical process consisting of oxidative roasting, acid leaching, and electrowinning (Li et al., 2012; Çoruh & Ergun, 2010). Zinc leaching residue (ZLR) consisting of zinc ferrite (ZnFe_2O_4) can easily be formed in large amounts during this process, and the formed ZLR is refractory to a traditional acid leaching process (Yan et al., 2014). Commonly, silver-bearing species are contained in ZLR and the silver-bearing ZLR is abandoned as valueless waste after target metals are totally recovered. In addition, the abandoned silver-bearing ZLR can cause serious environmental problems. Silver-bearing waste firebrick produced through smelting is also a type of important silver-bearing solid waste whose treatment or use is an important issue. In this work, two types of abandoned silver-bearing solid waste, ZLR and firebrick, were used for silver catalysis to promote chalcopyrite bioleaching. Silver was subsequently recovered from the bioleaching residue. This work is potentially useful to provide a promising technique to promote chalcopyrite bioleaching and for the comprehensive use of silver-bearing solid waste.

The pure chalcopyrite sample was obtained from the geological museum of Hunan Province of China. X-ray diffraction analysis showed that it was of high purity. The ZLR and waste firebrick were achieved at the smelting plant in Chenzhou, Hunan Province, China. The main mineralogical components of ZLR were silicate and zinc ferrite, and the main mineralogical components of waste firebrick were silicate and lead. A chemical element analysis (Tables 6.4 and 6.5) showed that the content of silver in ZLR and waste firebrick was about 294 and 358 g/t, respectively.

6.2.1 Comprehensive process

The comprehensive process is provided in Fig. 6.14. Silver-bearing solid waste (ZLR or waste firebrick) was added to the bioleaching system of chalcopyrite to promote chalcopyrite dissolution and the bioleaching residue was subsequently leached by thiourea to recover silver.

TABLE 6.4 The chemical elements analysis of zinc leaching residue (ZLR).

Element	O	Na	Al	Si	P	S	Cl	Ca
Content %	33.089	0.748	0.764	2.082	0.018	6.007	0.113	1.924
Element	Ti	Cr	Mn	Fe	Cu	Zn	As	Sr
Content %	0.054	0.016	1.627	23.449	0.203	22.940	1.650	0.510
Element	Ag	Cd	In	Sn	Sb	I	Pb	Bi
Content %	0.0294	0.347	0.099	0.346	0.182	0.025	2.895	0.283

TABLE 6.5 Chemical element analysis of waste firebrick.

Element	O	Na	Mg	Al	Si	P	S	Cl	K
Content %	27.5	0.11	23.74	2.93	1.66	0.292	0.242	0.172	0.059
Element	Ca	Ti	V	Cr	Mn	Fe	Co	Ni	Cu
Content %	1.1	0.0724	0.025	4.606	0.061	3.237	0.0139	0.0729	1.446
Element	Zn	As	Rb	Sr	Ag	Sb	W	Pb	
Content %	0.0495	0.688	0.0211	0.007	0.0358	1.35	0.0697	21.25	

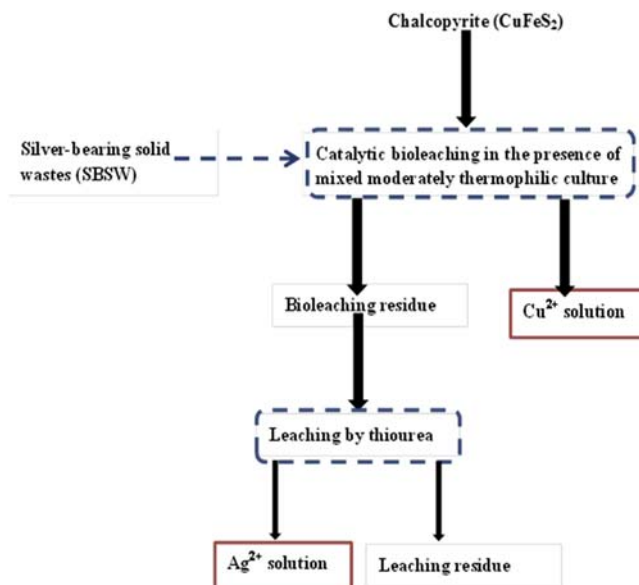


FIGURE 6.14 Technological process used in this work. From Wang, J., Liao, R., Tao, L., Zhao, H., Zhai, R., Qin, W., & Qiu, G. (2017). A comprehensive utilization of silver-bearing solid wastes in chalcopyrite bioleaching. *Hydrometallurgy*, 169, 152–157. <https://doi.org/10.1016/j.hydromet.2017.01.006>.

6.2.2 Recovery of Cu in bioleaching

Mixed moderately thermophilic cultures containing *Acidithiobacillus caldus* (CCTC AB 206240) and *L. ferriphilum* (CCTC AB 206239) were initially achieved from the Key Lab of Biohydrometallurgy of Ministry of Education, Central South University, Changsha, China. Mixed moderately thermophilic cultures were cultured in basic culture medium supplemented with 44.7 g/L ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) and 10 g/L sulfur as the energy sources, and then they were domesticated in the presence of 2% (wt%) chalcopyrite samples. The basic culture medium consisted of $(\text{NH}_4)_2\text{SO}_4$ (3.0 g/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 g/L), K_2HPO_4 (0.5 g/L), KCl (0.1 g/L), and $\text{Ca}(\text{NO}_3)_2$ (0.01 g/L). Mixed cultures obtained through filtration, centrifugation, and washing were inoculated into a 3-L beaker containing 2 L sterilized sulfuric acid solution, 40 g pure chalcopyrite sample, and 40 g silver-bearing solid waste (ZLR or waste

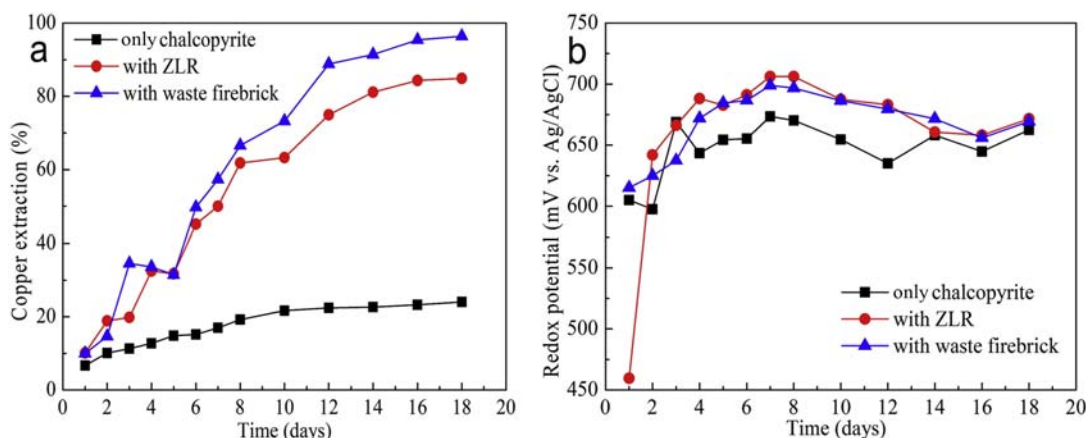


FIGURE 6.15 Bioleaching of chalcopyrite in the presence or absence of silver-bearing solid wastes (A) Copper extractions, (B) Redox potentials. ZLR, zinc leaching residue. From Wang, J., Liao, R., Tao, L., Zhao, H., Zhai, R., Qin, W., & Qiu, G. (2017). A comprehensive utilization of silver-bearing solid wastes in chalcopyrite bioleaching. *Hydrometallurgy*, 169, 152–157. <https://doi.org/10.1016/j.hydromet.2017.01.006>.

firebrick). The initial cell concentrations were all higher than 1.0×10^7 cells/mL. Bioleaching experiments were conducted at 170 rpm and 45°C, and the pH was maintained at around 1.70 with dilute sulfuric acid. Cu^{2+} concentrations were determined with an inductively coupled plasma-atomic emission spectrometer (ICP-AES) (America Baird Co. PS-6) and ORP were monitored by Pt electrode with reference to an Ag/AgCl electrode (3.0 mol/L KCl) (BPP-922). Solid samples were analyzed by XRD (DX-2700) and a scanning electron microscope (SEM) (JSM-6360 LV). In this work, all potential values were compared with the Ag/AgCl reference electrode (versus Ag/AgCl).

Fig. 6.15 shows that the addition of ZLR and waste firebrick increased the copper extractions from about 25% to more than 85% and 95%, respectively. Hence, the chosen ZLR and waste firebrick both greatly promoted chalcopyrite bioleaching.

6.2.3 Recovery of Ag in thiourea leaching

Bioleaching residues obtained through bioleaching were subsequently leached by thiourea. Thiourea leaching experiments were conducted in 50-mL shake flasks for 4.5 h at a liquid–solid ratio of 10:1, pH 1.0, 45°C, and thiourea concentration of 0.1–0.5 mol/L. The thiourea leaching experiments were all conducted in the absence of Fe^{3+} . Silver extractions were determined by ICP-AES (America Baird Co. PS-6).

As shown in Fig. 6.16, chalcopyrite almost completely dissolved in the presence of silver-bearing solid waste (ZLR and waste firebrick). In addition, a large amount of jarosite formed in the bioleaching residues.

SEM analysis (Fig. 6.17) indicated that the formed jarosite had the morphology of loose and porous flocules. Hence, roasting was not needed to destroy the structure of jarosite in the subsequent silver recovery process. Chemical phase analysis was used to analyze the contents and distribution of silver-containing species in silver-bearing solid waste by analysis center. First, an ethanol solution was used to extract silver from silver sulfate (Ag_2SO_4);

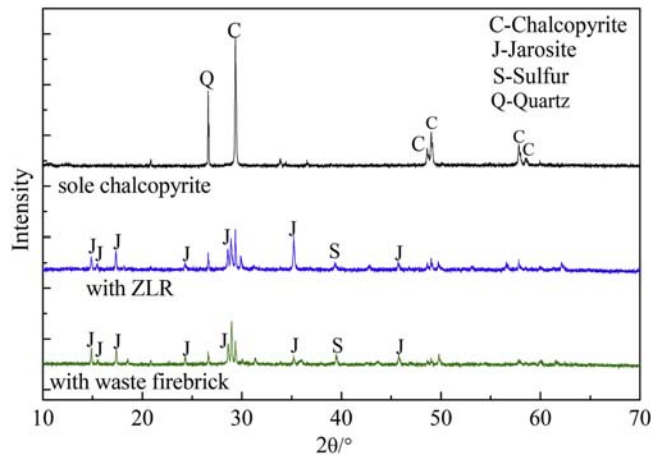


FIGURE 6.16 X-ray diffraction analysis of bioleaching residues. ZLR, zinc leaching residue. From Wang, J., Liao, R., Tao, L., Zhao, H., Zhai, R., Qin, W., & Qiu, G. (2017). A comprehensive utilization of silver-bearing solid wastes in chalcopyrite bioleaching. *Hydrometallurgy*, 169, 152–157. <https://doi.org/10.1016/j.hydromet.2017.01.006>.

Time	2 nd day	8 th day	16 th day
Condition			
Sole chalcopyrite			
With ZLR			
With waste firebrick			

FIGURE 6.17 Scanning electron microscope analysis of samples leached on different days. ZLR, zinc leaching residue. From Wang, J., Liao, R., Tao, L., Zhao, H., Zhai, R., Qin, W., & Qiu, G. (2017). A comprehensive utilization of silver-bearing solid wastes in chalcopyrite bioleaching. *Hydrometallurgy*, 169, 152–157. <https://doi.org/10.1016/j.hydromet.2017.01.006>.

TABLE 6.6 Distribution of silver-bearing species in silver-bearing solid waste.

Phase content (g/t)	Zinc leaching residue		Waste firebrick	
	Before bioleaching	After bioleaching	Before bioleaching	After bioleaching
Silver oxide	1.15	0.38	1.2	0.3
Metallic silver	182.05	140.32	283.44	168.31
Silver chloride	1.98	4.72	4.35	1.5
Silver sulfide	70.29	3.77	52.17	6.5
Iron minerals	36.81	9.1	12.74	10
Silicate minerals	2.38	4.1	4.78	5.78
Total	294.66	162.39	358.68	192.39

then, an acetic acid solution, ferric nitrate-mercuric oxide solution, ammonia solution, and sulfuric acid-thiourea solution were used to dissolve silver sulfate (Ag_2SO_4), silver oxide (Ag_2O), metallic silver (Ag), silver chloride (AgCl), and silver sulfide (Ag_2S), respectively. Finally, the residue was initially roasted with an electric resistance furnace and then leached by hydrofluoric acid to extract silver from silicate minerals. Nitric acid-sulfuric acid was used to remove organics and the obtained dissolved silver was analyzed by ICP-AES (America Baird Co. PS-6).

Table 6.6 shows that silver was distributed in metallic silver and silver sulfide in both ZLR and waste firebrick. The percentage of silver sulfide significantly decreased after bioleaching because silver sulfide can be oxidized during bioleaching. Silver extraction in the thiourea leaching of residue (ZLR), bioleached ZLR, waste firebrick, and bioleached waste firebrick in the presence of different thiourea concentrations is provided in Fig. 6.18. Silver extraction of bioleached ZLR was significantly higher than that of untreated ZLR when the thiourea concentration was low (less than 0.3 mol/L), and a final silver recovery of about 90% was achieved from the bioleached ZLR. The silver extraction of bioleached waste firebrick was remarkably higher than that of untreated waste firebrick, and a final silver recovery of about 90% was obtained from the bioleached waste firebrick. Therefore, silver was more easily extracted from bioleaching residues than untreated silver-bearing solid waste, and a high silver recovery of about 90% was finally achieved.

6.3 Summary

Ag^+ can greatly catalyze the dissolution process of chalcopyrite in a sulfuric acid-ferric sulfate system with the necessary presence of oxidants. DFT calculations indicate that the Cu-Fe hollow site of the chalcopyrite surface showed a significant adsorption interaction with Ag^+ ions and the possible formation of silver sulfide species. In addition, the reaction energetics for Ag^+ ion catalysis of chalcopyrite dissolution on the (001)-S and (112)-S surfaces are different, but the last step of Ag^+ ion diffusion is the same rate-limiting step with similar

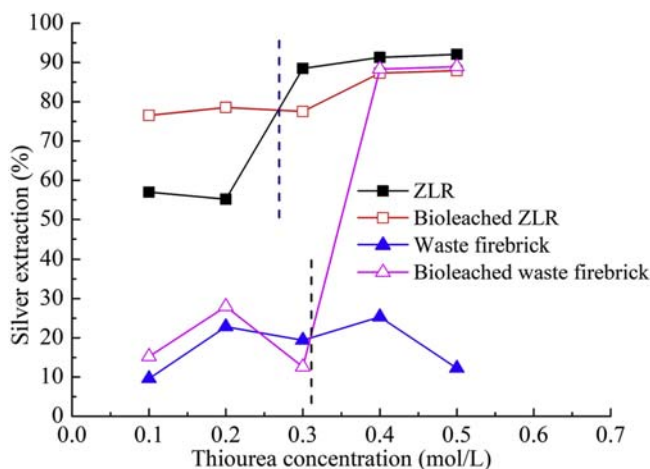


FIGURE 6.18 Thiourea leaching of zinc leaching residue (ZLR), bioleached ZLR, waste firebrick, and bioleached waste firebrick in the presence of different thiourea concentrations. From Wang, J., Liao, R., Tao, L., Zhao, H., Zhai, R., Qin, W., & Qiu, G. (2017). A comprehensive utilization of silver-bearing solid wastes in chalcopyrite bioleaching. *Hydrometallurgy*, 169, 152–157. <https://doi.org/10.1016/j.hydromet.2017.01.006>.

energy barriers of 1.19 and 1.06 eV, respectively. The adsorption of Ag^+ produced a sulfur vacancy and silver sulfides, which increased the electrochemical reactivity of chalcopyrite, such as low OCP, low resistance, and a high corrosion current density value. As a consequence, the electrochemical reactions of chalcopyrite are enhanced and the production of the passivation layer slows, catalyzing the oxidative dissolution process of chalcopyrite.

Abandoned silver-bearing solid waste of ZLR and firebrick were used as silver catalysis to promote chalcopyrite bioleaching. Silver was efficiently recovered from the bioleaching residue through thiourea leaching. Adding silver-bearing solid waste greatly accelerated chalcopyrite bioleaching although the redox potential always existed in the passivation region. Silver was more easily extracted from bioleaching residues than from untreated silver-bearing solid waste, and high silver recovery was achieved. This technique is promising for promoting chalcopyrite bioleaching and the comprehensive use of silver-bearing solid waste in industrial production.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (51704331), Young Elite Scientists Sponsorship Program by CAST (2017QNRC001), National Basic Research Program of China (Grant No. 2010CB630905), and High-Tech Research and Development Program of China (Grant No. 2012AA061501).

References

- Abdollahi, H., Noaparast, M., Shafaei, S. Z., Manafi, Z., Muñoz, J. A., & Tuovinen, O. H. (2015). Silver-catalyzed bioleaching of copper, molybdenum and rhenium from a chalcopyrite-molybdenite concentrate. *International Biodegradation and Biodegradation*, 104, 194–200. <https://doi.org/10.1016/j.ibiod.2015.05.025>.

- Abdollahi, H., Shafaei, S. Z., Noaparast, M., Manafi, Z., Niemelä, S. I., & Tuovinen, O. H. (2014). Mesophilic and thermophilic bioleaching of copper from a chalcopyrite-containing molybdenite concentrate. *International Journal of Mineral Processing*, 128, 25–32. <https://doi.org/10.1016/j.minpro.2014.02.003>.
- Ahmadi, A., Schaffie, M., Manafi, Z., & Ranjbar, M. (2010). Electrochemical bioleaching of high grade chalcopyrite flotation concentrates in a stirred bioreactor. *Hydrometallurgy*, 104(1), 99–105. <https://doi.org/10.1016/j.hydromet.2010.05.001>.
- Ahonen, L., & Tuovinen, O. H. (1990). Catalytic effects of silver in the microbiological leaching of finely ground chalcopyrite-containing ore materials in shake flasks. *Hydrometallurgy*, 24(2), 219–236. [https://doi.org/10.1016/0304-386X\(90\)90088-J](https://doi.org/10.1016/0304-386X(90)90088-J).
- Álvarez-Corral, M., Muñoz-Dorado, M., & Rodríguez-García, I. (2008). Silver-mediated synthesis of heterocycles. *Chemical Reviews*, 108(8), 3174–3198. <https://doi.org/10.1021/cr078361l>.
- Blöchl, P. E. (1994). Projector augmented-wave method. *Physical Review B*, 17953–17979. <https://doi.org/10.1103/physrevb.50.17953>.
- Chen, S. Y., & Lin, J. G. (2009). Enhancement of metal bioleaching from contaminated sediment using silver ion. *Journal of Hazardous Materials*, 161(2–3), 893–899. <https://doi.org/10.1016/j.jhazmat.2008.04.035>.
- Córdoba, E. M., Muñoz, J. A., Blázquez, M. L., González, F., & Ballester, A. (2008). Leaching of chalcopyrite with ferric ion. Part II: Effect of redox potential. *Hydrometallurgy*, 93(3–4), 88–96. <https://doi.org/10.1016/j.hydromet.2008.04.016>.
- Çoruh, S., & Ergun, O. N. (2010). Use of fly ash, phosphogypsum and red mud as a liner material for the disposal of hazardous zinc leach residue waste. *Journal of Hazardous Materials*, 173(1–3), 468–473. <https://doi.org/10.1016/j.jhazmat.2009.08.108>.
- Deng, T., & Liao, M. (2002). Gold recovery enhancement from a refractory flotation concentrate by sequential bioleaching and thiourea leach. *Hydrometallurgy*, 63(3), 249–255. [https://doi.org/10.1016/S0304-386X\(01\)00226-2](https://doi.org/10.1016/S0304-386X(01)00226-2).
- Fujisawa, M., Suga, S., Mizokawa, T., Fujimori, A., & Sato, K. (1994). Electronic structures of CuFeS_2 and $\text{Cu}_{10.9}\text{Fe}_{0.1}\text{S}_2$ studied by electron and optical spectroscopies. *Physical Review B*, 49(11), 7155–7164. <https://doi.org/10.1103/PhysRevB.49.7155>.
- Gahremaninezhad, A., Dixon, D. G., & Asselin, E. (2012). Kinetics of the ferric-ferrous couple on anodically passivated chalcopyrite (CuFeS_2) electrodes. *Hydrometallurgy*, 125–126, 42–49. <https://doi.org/10.1016/j.hydromet.2012.05.004>.
- Gahremaninezhad, A., Dixon, D. G., & Asselin, E. (2013). Electrochemical and XPS analysis of chalcopyrite (CuFeS_2) dissolution in sulfuric acid solution. *Electrochimica Acta*, 87, 97–112. <https://doi.org/10.1016/j.electacta.2012.07.119>.
- Gahremaninezhad, A., Radzinski, R., Gheorghiu, T., Dixon, D. G., & Asselin, E. (2015). A model for silver ion catalysis of chalcopyrite (CuFeS_2) dissolution. *Hydrometallurgy*, 155, 95–104. <https://doi.org/10.1016/j.hydromet.2015.04.011>.
- Gómez, E., Ballester, A., Blázquez, M. L., & González, F. (1999). Silver-catalysed bioleaching of a chalcopyrite concentrate with mixed cultures of moderately thermophilic microorganisms. *Hydrometallurgy*, 37–46. [https://doi.org/10.1016/S0304-386X\(98\)00070-X](https://doi.org/10.1016/S0304-386X(98)00070-X).
- Guo, P., Zhang, G., Cao, J., Li, Y., Fang, Z., & Yang, C. (2011). Catalytic effect of Ag^+ and Cu^{2+} on leaching realgar (As_2S_2). *Hydrometallurgy*, 106(1–2), 99–103. <https://doi.org/10.1016/j.hydromet.2010.12.006>.
- Hackl, R. P., Dreisinger, D. B., Peters, E., & King, J. A. (1995). Passivation of chalcopyrite during oxidative leaching in sulfate media. *Hydrometallurgy*, 39(1–3), 25–48. [https://doi.org/10.1016/0304-386X\(95\)00023-A](https://doi.org/10.1016/0304-386X(95)00023-A).
- Hiroyoshi, N., Arai, M., Miki, H., Tsunekawa, M., & Hirajima, T. (2002). A new reaction model for the catalytic effect of silver ions on chalcopyrite leaching in sulfuric acid solutions. *Hydrometallurgy*, 63(3), 257–267. [https://doi.org/10.1016/S0304-386X\(01\)00228-6](https://doi.org/10.1016/S0304-386X(01)00228-6).
- Hiroyoshi, N., Tsunekawa, M., Okamoto, H., Nakayama, R., & Kuroiwa, S. (2008). Improved chalcopyrite leaching through optimization of redox potential. *Canadian Metallurgical Quarterly*, 253–258. <https://doi.org/10.1179/000844308794408344>.
- Hohenberg, P., & Kohn, W. (1964). Inhomogeneous electron gas. *Physical Review*, 136(3B), B864–B871. <https://doi.org/10.1103/PhysRev.136.B864>.
- Kadrgulov, R. F., Yakshibaev, R. A., & Khasanov, M. A. (2001). Phase relations, ionic transport and diffusion in the alloys of Cu_2S - Ag_2S mixed conductors. *Ionics*, 7(1–2), 156–160. <https://doi.org/10.1007/BF02375484>.

- Khoshkhoo, M., Dopson, M., Shchukarev, A., & Sandström, Å. (2014). Chalcopyrite leaching and bioleaching: An X-ray photoelectron spectroscopic (XPS) investigation on the nature of hindered dissolution. *Hydrometallurgy*, 149, 220–227. <https://doi.org/10.1016/j.hydromet.2014.08.012>.
- Klauber, C. (2008). A critical review of the surface chemistry of acidic ferric sulphate dissolution of chalcopyrite with regards to hindered dissolution. *International Journal of Mineral Processing*, 86(1–4), 1–17. <https://doi.org/10.1016/j.minpro.2007.09.003>.
- Kohn, W., & Sham, L. J. (1965). Self-consistent equations including exchange and correlation effects. *Physical Review*, A1133–A1138. <https://doi.org/10.1103/physrev.140.a1133>.
- Kresse, G., & Furthmüller, J. (1996). Efficient iterative schemes for ab initio total-energy calculations using a plane-wave basis set. *Physical Review B: Condensed Matter and Materials Physics*, 54(16), 11169–11186. <https://doi.org/10.1103/PhysRevB.54.11169>.
- Kresse, G., & Joubert, D. (1999). From ultrasoft pseudopotentials to the projector augmented-wave method. *Physical Review B*, 59(3).
- Li, A., & Huang, S. (2011). Comparison of the electrochemical mechanism of chalcopyrite dissolution in the absence or presence of *Sulfolobus metallicus* at 70°C. *Minerals Engineering*, 24(13), 1520–1522. <https://doi.org/10.1016/j.mineng.2011.08.009>.
- Linge, H. G. (1976). A study of chalcopyrite dissolution in acidic ferric nitrate by potentiometric titration. *Hydrometallurgy*, 2(1), 51–64. [https://doi.org/10.1016/0304-386X\(76\)90013-X](https://doi.org/10.1016/0304-386X(76)90013-X).
- Li, M., Peng, B., Chai, L., Peng, N., Yan, H., & Hou, D. (2012). Recovery of iron from zinc leaching residue by selective reduction roasting with carbon. *Journal of Hazardous Materials*, 237–238, 323–330. <https://doi.org/10.1016/j.jhazmat.2012.08.052>.
- Miller, J., McDonough, & Portillo, H. (1981). *Electrochemistry in silver catalyzed ferric sulfate leaching of chalcopyrite, process and fundamental considerations of selected hydrometallurgical systems*.
- Monkhorst Hendrik, J., & Pack James, D. (1976). Special points for Brillouin-zone integrations. *Physical Review B*, 5188–5192. <https://doi.org/10.1103/physrevb.13.5188>.
- Muñoz, J. A., Dreisinger, D. B., Cooper, W. C., & Young, S. K. (2007). Silver-catalyzed bioleaching of low-grade copper ores. Part II: Stirred tank tests. *Hydrometallurgy*, 88(1–4), 19–34. <https://doi.org/10.1016/j.hydromet.2007.01.007>.
- Muñoz, J. A., Gómez, C., Ballester, A., Blázquez, M. L., González, F., & Figueroa, M. (1998). Electrochemical behaviour of chalcopyrite in the presence of silver and *Sulfolobus* bacteria. *Journal of Applied Electrochemistry*, 28(1), 49–56. <https://doi.org/10.1023/A:1003245701307>.
- Mustapha, A.-C., Mehmet, C., & Daniel, S. (1977). Oxydation ménagée de la chalcopyrite en solution acide: Analyse cinétique des réactions. II. Modèles diffusionnels. *Bulletin de La Société Française de Minéralogie et de Cristallographie*, 161–177. <https://doi.org/10.3406/bulmi.1977.7133>.
- Nakai, I., Sugitani, Y., Nagashima, K., & Niwa, Y. (1978). X-ray photoelectron spectroscopic study of copper minerals. *Journal of Inorganic and Nuclear Chemistry*, 40(5), 789–791. [https://doi.org/10.1016/0022-1902\(78\)80152-3](https://doi.org/10.1016/0022-1902(78)80152-3).
- Naodovic, M., & Yamamoto, H. (2008). Asymmetric silver-catalyzed reactions. *Chemical Reviews*, 108(8), 3132–3148. <https://doi.org/10.1021/cr068413r>.
- Nasluzov, V., Shor, A., Romanchenko, A., Tomashevich, Y., & Mikhlin, Y. (2019). DFT + U and low-temperature XPS studies of Fe-depleted chalcopyrite (CuFeS₂) surfaces: A focus on polysulfide species. *Journal of Physical Chemistry C*, 123(34), 21031–21041. <https://doi.org/10.1021/acs.jpcc.9b06127>.
- Nazari, G., Dixon, D. G., & Dreisinger, D. B. (2012). The mechanism of chalcopyrite leaching in the presence of silver-enhanced pyrite in the GalvanoxTM process. *Hydrometallurgy*, 113–114, 122–130. <https://doi.org/10.1016/j.hydromet.2011.12.011>.
- Palacio, C., Ocón, P., Herrasti, P., Diaz, D., & Arranz, A. (2003). XPS and ARXPS study of silver underpotential deposition on platinum in acid solution. *Journal of Electroanalytical Chemistry*, 53–58. [https://doi.org/10.1016/s0022-0728\(03\)00105-0](https://doi.org/10.1016/s0022-0728(03)00105-0).
- Parker, A., Klauber, C., Koungianos, A., Watling, H. R., & Van Bronswijk, W. (2003). An X-ray photoelectron spectroscopy study of the mechanism of oxidative dissolution of chalcopyrite. *Hydrometallurgy*, 71(1–2), 265–276. [https://doi.org/10.1016/S0304-386X\(03\)00165-8](https://doi.org/10.1016/S0304-386X(03)00165-8).
- Parker, A. J., Paul, R. L., & Power, G. P. (1981). Electrochemistry of the oxidative leaching of copper from chalcopyrite. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, 305–316. [https://doi.org/10.1016/s0022-0728\(81\)80549-9](https://doi.org/10.1016/s0022-0728(81)80549-9).

- Perdew, J. P., Burke, K., & Ernzerhof, M. (1996). Generalized gradient approximation made simple. *Physical Review Letters*, 77(18), 3865–3868. <https://doi.org/10.1103/PhysRevLett.77.3865>.
- Petersen, J., & Dixon, D. G. (2006). Competitive bioleaching of pyrite and chalcopyrite. *Hydrometallurgy*, 83(1–4), 40–49. <https://doi.org/10.1016/j.hydromet.2006.03.036>.
- Price, D. W., & Warren, G. W. (1986). The influence of silver ion on the electrochemical response of chalcopyrite and other mineral sulfide electrodes in sulfuric acid. *Hydrometallurgy*, 15(3), 303–324. [https://doi.org/10.1016/0304-386X\(86\)90063-0](https://doi.org/10.1016/0304-386X(86)90063-0).
- Sandström, A., Shchukarev, A., & Paul, J. (2005). XPS characterisation of chalcopyrite chemically and bio-leached at high and low redox potential. *Minerals Engineering*, 18(5), 505–515. <https://doi.org/10.1016/j.mineng.2004.08.004>.
- Sato, H., Nakazawa, H., & Kudo, Y. (2000). Effect of silver chloride on the bioleaching of chalcopyrite concentrate. *International Journal of Mineral Processing*, 59(1), 17–24. [https://doi.org/10.1016/S0301-7516\(99\)00061-7](https://doi.org/10.1016/S0301-7516(99)00061-7).
- Shirley, D. A. (1972). High-resolution x-ray photoemission spectrum of the valence bands of gold. *Physical Review B*, 5(12), 4709–4714. <https://doi.org/10.1103/PhysRevB.5.4709>.
- Tapera, T., Sheean, J., & Nikoloski, A. N. (2018). The effect of silver on the acidic ferric sulfate leaching of primary copper sulfides under recycle solution conditions observed in heap leaching. Part 2: Synergistic additives. *Hydrometallurgy*, 179, 1–7. <https://doi.org/10.1016/j.hydromet.2018.05.020>.
- Wang, M., Zhang, Y., Deng, T., & Wang, K. (2004). Kinetic modeling for the bacterial leaching of chalcopyrite catalyzed by silver ions. *Minerals Engineering*, 17(7–8), 943–947. <https://doi.org/10.1016/j.mineng.2003.11.021>.
- Weibel, J. M., Blanc, A., & Pale, P. (2008). Ag-mediated reactions: Coupling and heterocyclization reactions. *Chemical Reviews*, 108(8), 3149–3173. <https://doi.org/10.1021/cr078365q>.
- Yan, H., Chai, L. Y., Peng, B., Li, M., Peng, N., & Hou, D. K. (2014). A novel method to recover zinc and iron from zinc leaching residue. *Minerals Engineering*, 55, 103–110. <https://doi.org/10.1016/j.mineng.2013.09.015>.
- Yang, Y., Harmer, S., & Chen, M. (2015). Synchrotron-based XPS and NEXAFS study of surface chemical species during electrochemical oxidation of chalcopyrite. *Hydrometallurgy*, 156, 89–98. <https://doi.org/10.1016/j.hydromet.2015.05.011>.
- Yuehua, H., Guanzhou, Q., Jun, W., & Dianzuo, W. (2002). The effect of silver-bearing catalysts on bioleaching of chalcopyrite. *Hydrometallurgy*, 64(2), 81–88. [https://doi.org/10.1016/S0304-386X\(02\)00015-4](https://doi.org/10.1016/S0304-386X(02)00015-4).

This page intentionally left blank

Industrial application for chalcopyrite bioleaching

7.1 Bioleaching technology

A long time ago, biohydrometallurgical/bioleaching technology was used to extract Cu. The use of related technologies in China may be traced back to 100–200 BCE, although people at that time were not clear about the underlying principles (Needham et al., 1976; Rossi, 1990). In the 6th or 7th century BCE, a Chinese work of literature called *San Hai Jing* recorded that the Luo Shui river flows through the SongGuo mountain and into the WeiShui river, resulting in copper production. In the Han Dynasty, Liu An, King of Huainan, wrote a book titled *Huai Nan Wan Bi Shu* (in the 2nd century BCE) and recorded copper recovered from an aqueous solution using metallic iron as a reductant. In the Tang and Song Dynasties (approximately CE 600–960), hydrometallurgical factories for copper were established, and the highest copper production reached more than 500,000 kg annually. After CE 200, similar technologies occurred in parts of West Asia and Europe. However, it is unclear whether the technology originated from China or elsewhere. Although the origin of biohydrometallurgical technology to extract copper is early, it was not until the mid-20th century that the specific microorganism species involved in copper leaching systems were identified (Ehrlich, 2001). In 1947, Colmer and Hinkle (Colmer & Hinkle, 1947) first isolated one bacterial strain from the acid mine drainage; they found that the bacterial isolate had a significant role in the oxidation of Fe^{2+} and sulfide minerals. In the 1950s, biohydrometallurgy technology was applied in the Kennecott copper mine. Since then, this technology has been widely used to process secondary copper sulfide ores because of its simple operation, low cost, and environmentally friendly characteristics (Brierley & Brierley, 2001).

Conventional beneficiation-pyrometallurgy and bioleaching flowsheets are shown in Fig. 7.1. Chalcopyrite mainly exists in raw ores characterized by a low grade, fine, and complex distribution, and it is mainly treated using beneficiation-pyrometallurgy technology. Notably, biohydrometallurgy (bioleaching) could replace conventional beneficiation-pyrometallurgy in processing low-grade and fine and complex distributed ores, because the latter has the following characteristics: (1) in contrast to biohydrometallurgy, the

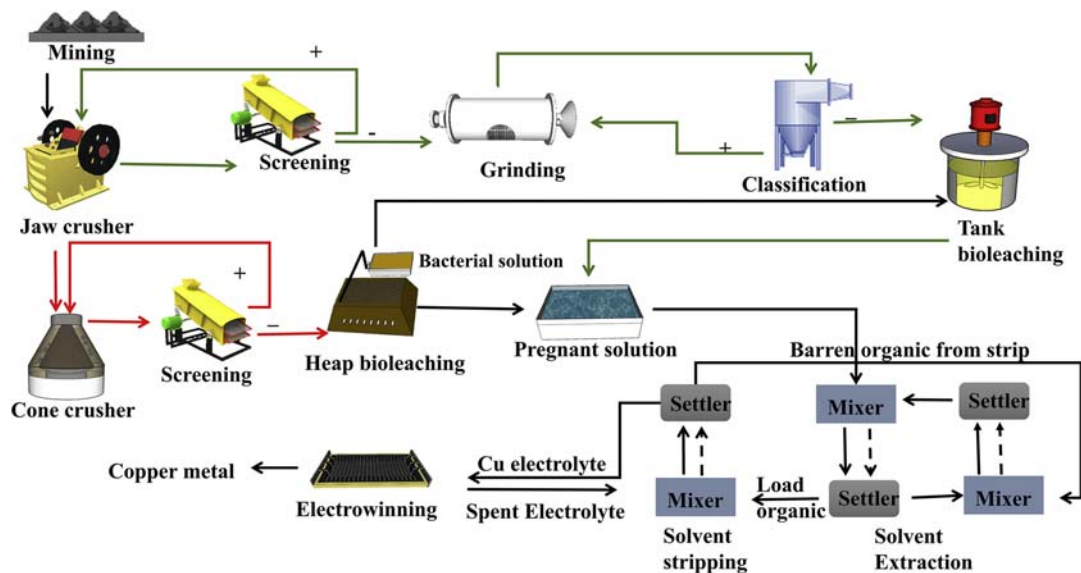


FIGURE 7.1 Schematic diagram of bioleaching of copper ores. From Zhao, H., Zhang, Y., Zhang, X., et al. *The dissolution and passivation mechanism of chalcopyrite in bioleaching: An overview*[J]. *Minerals Engineering*, 2019, 136: 140–154. <https://doi.org/10.1016/j.mineng.2019.03.014>.

pyrometallurgical process is often deteriorated by impurities in the copper concentrate; (2) the pyrometallurgical process can produce heavy metals containing fumes, dust, CO_2 , and SO_2 , resulting in serious air pollution. However, bioleaching mainly oxidizes sulfide minerals into elemental sulfur or sulfuric acid rather than SO_2 , which reduces the degree of air pollution and acid consumption (Fig. 7.2); (3) during the existing pyrometallurgical process, a massive dosage of flotation reagents such as activators, depressants, collectors, and frothers is needed, and recycling water in the beneficiation process is also a challenge, which causes serious water and soil contamination; (4) it is difficult to process those low-grade and fine and complex distributed ores economically and efficiently, owing to the high-energy consumption of the conventional beneficiation-pyrometallurgy technique (such as ball milling and high-temperature smelting).

In a previous study, the method of life cycle assessment was implemented to compare conventional beneficiation-pyrometallurgy and bioleaching technologies technoeconomically for the copper ores of Zijing Mining (Table 7.1) (Ruan et al., 2010). This study revealed that energy consumption, water consumption, CO_2 emissions, and SO_2 emissions from bioleaching technology are all significantly less than those caused by beneficiation-pyrometallurgy technology. Accordingly, bioleaching has been considered an important and clean technology for processing low-grade and complex ores, mainly because of its environmental and low-cost advantages (Anjum et al., 2012). Also, it was believed that bioleaching as a vital bioremediation technology may have a key role in processing solid waste and secondary resources (Akçil et al., 2015; Asghari et al., 2013; Erüst et al., 2013;

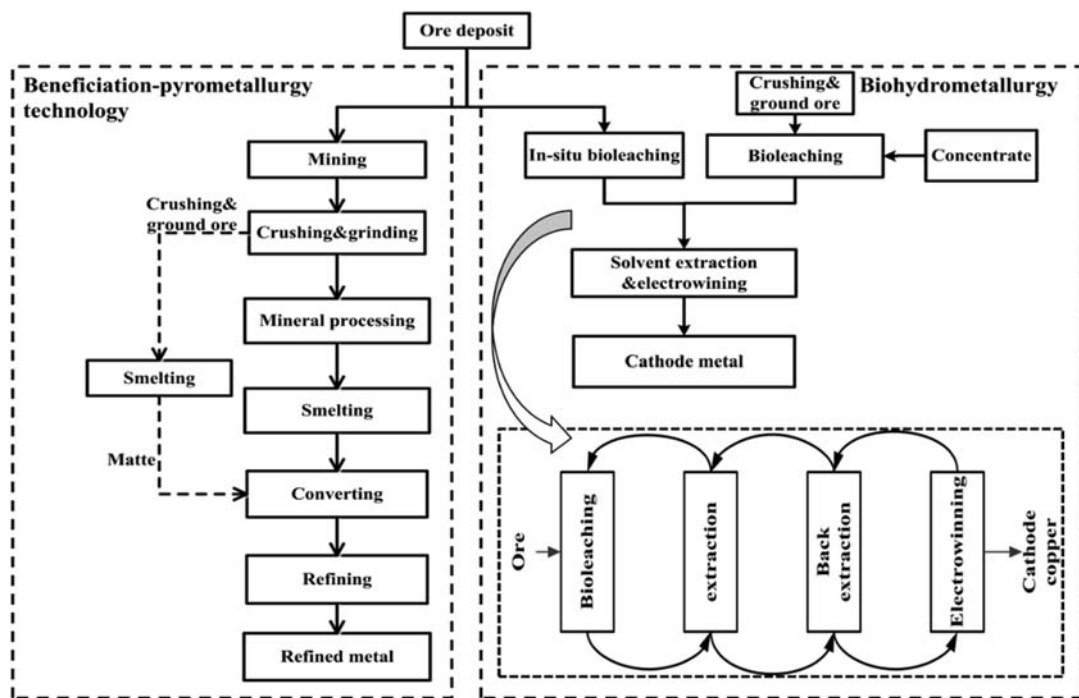


FIGURE 7.2 Flowsheet of conventional beneficiation-pyrometallurgy and bioleaching technology. From Zhao, H., Zhang, Y., Zhang, X., et al. The dissolution and passivation mechanism of chalcopyrite in bioleaching: An overview[J]. Minerals Engineering, 2019, 136: 140–154. <https://doi.org/10.1016/j.mineng.2019.03.014>.

TABLE 7.1 Comparative techno-economic analysis of conventional beneficiation-pyrometallurgy and bioleaching processes of copper ores of Zijing Mining using life cycle assessment (LCA^a) (Ruan et al., 2010).

Technology	Energy consumption		Water consumption/m ³	CO ₂ emissions/kg	SO ₂ emissions ^b /kg
	Electricity/kW·h	Coal/kg			
Bioleaching	3915.14	1402.22	21.76	4090.57	11.93
Beneficiation-pyrometallurgy	8706.9	3656.24	168.09	10,909.29	79.04
Relative amount ^c	44.97%	38.35%	12.85%	37.50%	15.09%

^a LCA: Life Cycle Assessment; The parameters show the resource consumption and pollutant emission caused by producing one ton of metal copper.

^b SO₂ emission: Complete bioleaching technology includes mining, crushing, bioleaching, extraction and electrowinning, etc. where SO₂ can be produced.

^c The "Relative amount" means the techno-economic parameter ratios of bioleaching processes to conventional beneficiation-pyrometallurgy. ("Relative amount" = 100% × "Bioleaching"/"Beneficiation-pyrometallurgy").

Hoque & Philip, 2011; Pant et al., 2012; Pathak et al., 2009; Watling et al., 2016), such as tailings, electronic waste, spent catalyst, sludge, sediment, fly ash, and smelter waste. In addition, its theory and technique may be extended to the other research areas of mineral

processing and extractive metallurgy, the biological environment, biomass conversion, biodeterioration, biogeochemistry, and biomineralization. In short, studies on biohydrometallurgy have important academic and practical value in the field of mining and metallurgical industry.

7.2 Industrial practice cases

Based on the BioCOP Process, BHP Billiton designed and operated the first commercial demonstration plant for bioleaching chalcopyrite concentrates using thermophilic microorganisms (Batty & Rorke, 2006). Nonetheless, large-scale commercial application of chalcopyrite bioleaching, especially heap bioleaching of chalcopyrite, has still developed slowly owing to the refractory nature of chalcopyrite. Some main industrial practice cases of copper bioleaching worldwide are summarized in Table 7.2.

7.3 Key parameters and controlling techniques

7.3.1 Redox potential/oxidation-reduction potential

The dissolution process of chalcopyrite in bioleaching depends on the redox potential/oxidation-reduction potential (ORP). More specially, a relatively low potential is beneficial to chalcopyrite dissolution, and a high ORP can easily cause chalcopyrite to become passivated (Gómez et al., 1996; Third et al., 2002). To explain how low potential causes the dissolution of chalcopyrite in bioleaching, some publications have provided answers. Hiroyoshi et al. (Hiroyoshi et al., 2000) put forward a two-step dissolution model to explain this. First, chalcopyrite was degraded into chalcocite (Cu_2S). Then, the formed chalcocite was rapidly dissolved. Our previous work (Zhao, Wang, Gan et al., 2015; Zhao, Wang, Yang et al., 2015) further supported this conclusion, and a mathematic model was proposed to predict the optimum redox potential in the chalcopyrite bioleaching system. This model revealed the relation between the optimum redox potential and the multiple factors of the bioleaching system, including the Cu^{2+} concentration, Fe^{2+} concentration, and temperature. Fe^{3+} is the main oxidant and determining factor for the redox potential in chalcopyrite bioleaching. Vilcaez et al. (Vilcáez et al., 2009; Vilcáez & Inoue, 2009) claimed that both cathodic reduction and anodic oxidation were involved in the leaching process of chalcopyrite. The dissolution process was mainly controlled by the Fe^{2+} concentration when the potential was low (648 mV versus Standard Hydrogen Electrode (SHE)), and chalcopyrite was first converted to Cu_2S , which could be quickly oxidized; chalcopyrite was directly oxidized by Fe^{3+} when the solution potential was high (>648 mV versus SHE), but the oxidizing effect was disappointing, resulting in low leaching efficiency.

Many efforts have been made to control the ORP at a suitable range to enhance the dissolution of chalcopyrite in bioleaching. Fe^{3+} is the main oxidizing agent in the chalcopyrite bioleaching system (Zhao et al., 2017). Thus, the ORP of chalcopyrite bioleaching system is mainly contributed by the redox couple of $\text{Fe}^{3+}/\text{Fe}^{2+}$. All kinds of methods can be experimented with to control the ORP by adding a new redox couple (oxidizing agent) or by adjusting the ratio of Fe^{3+} to Fe^{2+} indirectly, such as adding chemical agents and minerals, adjusting dissolved oxygen, and using electrochemical reactors and weak iron oxidizers.

TABLE 7.2 Industrial cases of copper bioleaching across the globe.

Typical mine	Location	Features	Minerals	References
Dexing copper mine	Dexing, Jiangxi Province, China	The stripping waste rock dump contained 1.2 million t total copper piled up at a height of 70 m, with an inclination slope angle of 55 degrees and an area of 7,570,000 m ² with about 600 million tons of waste rocks in total. The Cu grade of this ore is between 0.1% and 0.279%. However, all of the ore, waste rocks, and clay were mixed together during early dumping stage, and the average grade of this dump was 0.08%. The ore contains 0.45% primary copper sulfide, 0.028% secondary copper sulfide, 0.0068% free copper oxide, and 0.0052% bonded copper oxide. Dump bioleaching plant with annual production 2000 t was put into use in Oct. 1997; copper extraction rate annually is <10%. Microbial communities in leaching solution and leaching heap were dominated by <i>Acidithiobacillus</i> and <i>Leptospirillum</i> , suggesting that these microorganisms might be essential participants in the bioleaching process.	Chalcopyrite	Hu et al. (2015), Wu et al. (2009), Xiao et al. (2016), Yin et al. (2018), Zhang, Niu et al. (2016), Zhang, Wei et al. (2016)
Codelco mine of Chuquicamata	Chile	The BioCOP process developed by BHP Billiton employed acidophilic, iron-oxidizing archaea for bioleaching chalcopyrite concentrates in aerated stirred-tank reactor systems. The commercial demonstration plant, with a design production rate of 200,00 t copper per annum, was commissioned in 2003. The microbes used consisted of members of the genera <i>Sulfolobus</i> -like spp, <i>Metallosphaera</i> -like spp, and <i>Acidianus</i> -like spp. The process operated at 78°C, pH 1.5%, and 12% (w/w) pulp density with >98% copper recovery.	Chalcopyrite	Batty and Rorke (2006), Brierley (2008), Du Plessis et al. (2007)
Dos Amigos mine	Chile	Operations began early in 1997 producing 5000 t/y copper cathode, which was subsequently expanded in 1999 to a current 10,000 t/y. As a result, leaching performance has been successful, reaching 80% total copper recovery in a period of 300–350 days. The mineralogical composition of the ore is digenite (Cu ₉ S ₅), chalcocite (Cu ₂ S), and chalcopyrite (CuFeS ₂) as the only copper species, with digenite being dominant.	Digenite chalcocite chalcopyrite	Gericke et al. (2009), Olson et al. (2003), Du Plessis et al. (2007)
Sarcheshmeh copper complex	Kerman province, Iran	Mintek's high-temperature technology for chalcopyrite heap bioleaching has been successfully demonstrated in partnership with the National Iranian copper industries Company in Iran, where 14 20,000 t pilot heaps operated from 2005 to 2009. Temperatures of around 40°C were maintained in the heaps, with maxima of around 45°C observed. An acceptable rate of copper extraction could be maintained, while the formation of jarosite remained under control. The test heap material contained 0.6% copper, of which about 50% is in the form of chalcopyrite; the balance occurs essentially as chalcocite. In this bioheap system, mixed mesophilic and thermophilic microbes were employed to extract Cu.	Chalcopyrite chalcocite	Gericke et al. (2009), Reza et al. (2014)

Continued

TABLE 7.2 Industrial cases of copper bioleaching across the globe.—cont'd

Typical mine	Location	Features	Minerals	References
Escondida mine	Chile	In early 1999, a test work commenced in Escondida mine, where mesophile treatment was used for heap bioleaching of run-of-mine sulfide ore containing 0.3–0.7% copper. The tested ores contain low-grade copper sulfide minerals such as chalcocite, covellite, and chalcopyrite, as well as oxide and mixed oxide/sulfide ores. A 300,000-t trial heap at Escondida mine was successfully treated in 2004. These results of the test dump are consistent with expected recoveries of 60% for chalcocite, 45% for covellite, and 15% for chalcopyrite. The full commercial plant will produce 180,000 t copper cathode. The commercial heap will be constructed on a prepared, lined pad with piped solution collection, and forced air distribution. The heap base was expected to be 4.9×2.0 km, constructed in seven lifts of 18 m each up to a height of 126 m. In the bioleaching cycle, <i>Acidithiobacillus ferrooxidans</i> and <i>Sulfurisphaera</i> -like archaea were dominant within each respective phylogenetic domain at the first stage. At the second stage (from days 255 to 338), <i>Leptospirillum</i> and <i>Ferroplasma</i> groups were mainly detected, respectively. Finally (the third period from operation days 598 to 749), <i>Sulfobacillus</i> -like microorganisms became predominant, whereas <i>Ferroplasma</i> was the only archaea detected. The pH of feed solutions for both modules was controlled by adding sulfuric acid ranging from an approximate pH of 1.0 to 1.2.	Chalcocite covellite chalcopyrite	Clark et al. (2006), Demergasso et al. (2005), Gericke et al. (2009), Soto et al. (2013)
Chuquicamata mine	Chile	Very low-grade ore (0.3% total copper or less) has been mined at the Chuquicamata pit over several years; dump bioleaching of run-of-mine size material is the only economically feasible procedure for treatment. Copper recovery using this technique was originally projected at 25%, but it was reported to be below 15% of the total copper content. The solvent extraction–electrowinning units began operation in mid-1994 with a design production level of 12,500 t/y of copper cathode.	Chalcopyrite	Du Plessis et al. (2007)
Malanjkhand copper mine	Malanjkhand, Madhya Pradesh, India	Heap bioleaching of low-grade chalcopyrite (CuFeS_2) was carried out in 1000 t scale, made of a truncated cone with the dimensions: top area 86.1 m ² , base area 268.26 m ² , and height 2.8 m. Chemical analysis of the low-grade ore showed 0.34% Cu (0.33% as sulfide and <0.01% as oxides), 2.6% Fe, 0.82% S, and 90.27% acid-insoluble. The percentage of sulfide minerals present in the low-grade ore sample used in the heap had chalcopyrite (85%), bornite (4%), pyrite (8%), chalcocite (3%), and traces of malachite. The microorganisms used in the pilot scale were a mixed culture of acidophilic bacteria predominantly of the <i>A. ferrooxidans</i> strain. Leaching studies showed a cumulative copper dissolution rate of 0.14%/d (2.37 kg/d). Overall recovery of the heap was 30% over a 383 days.	Chalcopyrite bornite chalcocite malachite	Panda et al. (2012)

Zijinshan copper mine	Shanghang, Fujian Province, China	The Zijinshan copper mine is the largest chalcocite deposit in China. The attempt to recover copper from the ore by bioleaching began at the end of 1998. After metallurgical studies carried out over 2 years, a pilot plant consisting of bio-heap leaching and solvent extraction-electrowinning (SX–EW) was built at the Zijinshan copper mine at the end of 2000, with a production capacity of 300 t/a copper cathode. After its successful operation for 1.5 years, the plant was scaled up to a capacity of 1000 t/a copper cathode by Jun. 2002. The average copper grade of the stacked ores was decreased from 0.42% in 2006 to 0.34% in 2009, with an average grade of 0.38%. The overall heap area was 0.2 million square meters. The heap consists of three lifts, each with a height of 8–10 m. Acid mine water was adjusted to pH 1.7 by dilute sulfuric acid (2%) before irrigation into the heap. An irrigation pipe system was arranged in a 3 × 3 -m grid and sprinkler leaching was used with a solution irrigation rate around 12–16 L/m² h. The dominant group in the acid mine water inoculated into the system was mainly <i>Acidithiobacillus</i>. After inoculation, the microbial community evidently changed. In the startup stage (Jun. 2006), <i>Leptospirillum</i> was dominant, which accounted for 74% of the community, and <i>Ferroplasma</i> was the only detected archaea, which accounts for 6% of the whole prokaryotes. While at steady operation stage (May 2008), the proportion of genus <i>Leptospirillum</i> was sharply reduced (from 48.5% to 5%) from a higher depth to a lower depth, and an reverse correlation of increased <i>A. ferrooxidans</i> (from 0.95% to 15.00%) and <i>Sulfobacillus thermotolerans</i> (from undetectable to 7.00%) was found in the heap. Sulfur oxidizers including moderate thermophilic <i>Aspergillus albertensis</i> and <i>Acidithiobacillus caldus</i> vertically increased from a higher depth to a lower depth (from 50.47% to 70.00%). In the heap, <i>Acidithiobacillus</i> was mainly located in the inner and higher sites of ore heap. <i>Leptospirillum</i> was mainly located in the outer and lower sites of ore heap, and heterotrophic and facultative microbes were mainly located in the middle of the ore heap.	Chalcocite covellite enargite	Liu, Chen et al. (2016), Ruan et al. (2006), (2011)
Cerro Colorado mine	Chile	The bacterial leaching process was used at Cerro Colorado mine in 1993. Efforts to maximize biological activity in heap systems ended in the 1980s. Stand-alone bioheap leaching is relatively well-established at the Cerro Colorado mine; it commenced with a capacity of 130,000 t of higher-grade copper cathode annually in 2004. Cerro Colorado belongs to BHP Billiton and continues to operate with a dominant sulfide copper ore feed at a grade of a little less than 1% total copper. It obtains an average 84%–85% total copper recovery, equivalent to some 90%–95% of leachable copper, in a 450- to 500-day total leach period.	Chalcocite	Domic (2007), Gericke et al. (2009), Olson et al. (2003)

Continued

TABLE 7.2 Industrial cases of copper bioleaching across the globe.—cont'd

Typical mine	Location	Features	Minerals	References
Quebrada Blanca mine	Chile	The plant came online in 1994 with a design capacity of 75,000 t/y of copper cathode, but it has reached 80,000 t/y. The design target is to reach 80% copper recovery, which requires an extended leach period dependent on temperature. For the lower range of 15–18°C, the duration of leaching was up to 500 days. This period may shorten to 300–360 days if the temperature is kept at 22–25°C. Important improvements at Quebrada Blanca mine, such as forced aeration, have improved the process considerably, because aeration has positive effect on microbial activity and copper extraction.	Chalcocite	Domic (2007) , Gericke et al. (2009) , Olson et al. (2003)
Sociedad Minera Pudahuel Lo Aguirre mine	Santiago, Chile	The Lo Aguirre operation was built and commissioned in 1980. A nominal 15,000 t/y of higher-grade copper cathodes was produced from the time of startup until it was shut down in 2001 because of the total depletion of ore sources in the vicinity. The ore initially contained mainly copper oxide species, but over the years, a growing proportion of the feed consisted of chalcocite (Cu ₂ S), and in 1982, a significant secondary bioleach operation of initial tailings was initiated. By 1985, increased sulfide leach production justified the construction of an expansion of the solvent extraction plant to process the increased amount of lower copper tenor pregnant leach solutions more typical of a bioleaching process.	Chalcocite	Brierley (2008) , Domic (2007) , Olson et al. (2003)
Radio Hill mine	Australia	The Mt Sholl copper–nickel deposit, with grades 0.92% Cu, 0.67% Ni, 11.1% Fe 4.05% S, 2.3% Al, and 4.0% Al, is in the Pilbara region of Western Australia. Sulfide minerals make up about 15% by volume of the ore but are finely disseminated within the host rock. Pentlandite particles are in the range 30 µm to 2 mm, but chalcopyrite is 60% –70% < 100 µm, with more than 50% locked in unreactive silicate minerals. Two 5000-t ore heaps 5 m high were constructed at Radio Hill mine. The first heap was stacked with Mt Sholl disseminated ore (crush size passing 7.5 mm) and equipped with aeration and irrigation lines. The aeration rate was 1000 m ³ /h. The heap was irrigated with acidified pond water until the exit solution was pH <2.2. The moderately thermophilic microorganisms (contained <i>Sulfobacillus</i> and <i>Thermoplasma</i>) grown at 50 –55°C at pH 1.8 were inoculated via the irrigation system. The second heap was stacked with barren rock, 100% passing 50 mm, and subjected to an acid rinse, as earlier. This heap was inoculated with iron oxidizing bacteria to create an iron oxidation reactor, the solution from which passed to precipitation where ferric ion was controlled at <10 g/L in solution. A 90% recovery of nickel was achieved in just less than a year. Copper recovery lagged behind nickel and achieved only 50% recovery.	Pentlandite chalcopyrite	Watling (2008)

Talvivaara multimetal deposits	Finland	The complex ore, averaging 0.27% Ni, 0.56% Zn, and 0.14% Cu, contains pyrrhotite, pyrite, sphalerite, pentlandite, violarite, chalcopyrite and graphite. The 17,000-t demonstration heap was stacked $30 \times 60 \times 8$ m high, contained on three sides by a wall of boulders. Ore at 80% and passing 8 mm and <10% fines, was agglomerated with acid and a prepared inoculum. The heap was irrigated with a pH 1.8 solution at a rate of 5 L/m ² /h; solution temperatures ranged between 4°C and 20°C. Temperatures within the heap varied between 30°C and 90°C. The microbial community in the heap (monitored via leachate analyses) varied in the first few months and were then dominated by <i>At. ferrooxidans</i> and <i>Desulfotomaculum geothermicum</i> ; other species identified were <i>Thiomonas arsenivorans</i> , <i>Alicyclobacillus tolerans</i> , and <i>Ferromicrobium acidophilum</i> . Analyses of ore samples from the heap revealed <i>A. ferrooxidans</i> , <i>A. caldus</i> , and <i>F. acidophilum</i> . Recoveries were Ni 92%, Zn 82%, Co 14%, and copper 2% in 500 days.	Pyrrhotite pyrite sphalerite pentlandite violarite chalcopyrite graphite	Watling (2008)
Jinchuan copper–nickel mine	Jinchang, Gansu Province, China	A chemical analysis of ore samples from the heap revealed Ni 0.60%, Co 0.026%, Cu 0.30%, Fe 10.4%, S 2.2%, MgO 31%, CaO 0.80%, SiO ₂ 39%, and Al ₂ O ₃ 2.0%. Mineralogical examination of the ore sample showed that the main sulfide minerals were ~3.3% pyrrhotite (Fe _{1-x} S), ~1.6% pentlandite ((Fe, Ni) ₉ S ₈), ~0.8% chalcopyrite (CuFeS ₂), ~0.5% pyrite (FeS ₂), and ~0.2% violarite ((Fe, Ni) ₃ S ₄). A layer of support rock, low in carbonate and other acid-consuming minerals and sized at 10–20 mm, was first placed 100 mm deep in the bottom of the heap before the 500-t mineral ore sample was loaded. The 500 t of ore was stacked $5 \times 12 \times 4$ m high on the pad. Aeration pipes were located 1 m from the base and ran the length of the heap. The heap was covered and irrigated by a network of irrigation pipes and drippers. The microorganisms used in the test were a mixture of mesophiles, <i>A. ferrooxidans</i> (FJ164046; DQ321745), <i>Acidithiobacillus thiooxidans</i> (EF059760), and <i>Leptospirillum ferrooxidans</i> (DSM2391). The pH was 1.7–2.0 and the irrigation rate was 5–10 m ³ /h. Clean air was supplied via a rotameter and the air flow rate was 100 m ³ /h. A recovery of 84.6% Ni, 75.0% Co, and 32.6% Cu was achieved within 350 days of leaching, including 80 days of acid preleaching and 270 days of bioleaching.	Pyrrhotite pentlandite chalcopyrite pyrite violarite	Qin et al. (2009)
Gunpowder's Mammoth mine	Australia	Commenced with a capacity of 130,000 t of higher-grade copper cathode annually in 1991.	Chalcocite	Olson et al. (2003)
Mt Leyson	Australia	Commenced with a capacity of 1370 t/d of ore in 1992–97.	Chalcocite	Olson et al. (2003)

Continued

TABLE 7.2 Industrial cases of copper bioleaching across the globe.—cont'd

Typical mine	Location	Features	Minerals	References
Girilambone	Australia	Commenced with a capacity of 2000 t/d of ore in 1993–2003.	Chalcocite	Olson et al. (2003)
Ivan mine	Chile	The ore is primarily a mixture of oxides containing atacamite ($\text{Cu}_2\text{Cl}[\text{OH}]_3$) and chalcocite-type sulfide copper. Commenced with a capacity of 10,000 t of higher-grade copper cathode annually in 1994.	Atacamite chalcocite	Olson et al. (2003)
Andacollo	Chile	Commenced with a capacity of 10,000 t/d of ore in 1996.	Chalcocite	Olson et al. (2003)
Cerro Verde	Peru	Commenced with a capacity of 32,000 t/d of ore in 1996.	Chalcocite	Olson et al. (2003)
Zaldívar mine	Chile	Commenced with a capacity of ~20,000 t/d of ore in 1998.	Chalcocite	Olson et al. (2003)
S&K copper	Myanmar	Commenced with a capacity of 18,000 t/d of ore in 1998.	Chalcocite	Olson et al. (2003)
Equatorial Tonopah	United States	Commenced with a capacity of 24,500 t/d of ore in 2000–01.	Chalcocite	Olson et al. (2003)
Yangla copper mine	Diqing County, Yunnan Province, China	Alkaline bioleaching of low-grade oxide copper ores (copper oxide ore, total Cu 1.01%, with malachite 0.36%, chrysocolla 0.29%, and chalcocopyrite 0.29%) by <i>Providencia</i> sp. JAT-1; initial pH 8 and 30°C; Cu extraction rate was 54.5% after 156 h.	Malachite chrysocolla chalcocopyrite	Yin et al. (2018)
Guanfang copper mine	Lincang County, Yunnan Province, China	Bioheap leaching factory of primary copper sulfide and secondary copper sulfide (total Cu 0.9%) was built in 2003.	—	Yin et al. (2018)
Zhongtiaoshan copper mine	Yuncheng City, Shanxi Province, China	Cu 0.65%, in which secondary copper sulfide was 59.1%, free oxide copper 37.4%; and SiO_2 68.44%. In situ leaching; underground; bioleaching and acid leaching (extraction electrowinning process); >500 t/a in 2000.	—	Yin et al. (2018)
Tongguanshan copper mine	Tongling City, Anhui Province, China	Underground bioleaching since 1965; Cu recovery reached 95% in 1980; discontinued production in 2003; bioleaching tests from 1972 to 1980; total Cu 0.9%.	—	Yin et al. (2018)
Dabaoshan copper mine	Qujiang County, Guangdong Province, China	Total Cu 1.06%, in which primary and secondary copper sulfide occupied 90% of Cu, Fe 26.8%. Biodump leaching by <i>Thiobacillus ferrooxidans</i> (T. f.) obtained from Dabaoshan mining region	—	Yin et al. (2018)

Yulong copper mine	Jiangda County, Tibet Autonomous Region, China	Bioheap leaching of oxide and copper sulfide minerals (total Cu 2.75%, in which secondary copper sulfide was 28.95% and primary copper sulfides were 35%); high altitude (4569–5118 m) of Tibet; bioleaching SX-EW technology, realizing >80% copper extraction rate of sulfide ores.	—	Yin et al. (2018)
Asele copper mine	Habahe County, Xinjiang Autonomous Region, China	Total Cu 2.43%, Cu recovery reached 80%; formal operation of bioleaching industrial plant used since Jul. 2004.	—	Yin et al. (2018)
Yongping copper mine	Shangrao City, Jiangxi Province, China	Second largest open copper pit in China; formal operation from Oct. 1984; recycle low-grade ores and waste by bioleaching since the 1990s. Total Cu 0.32%, in which primary copper sulfide was 65.6% and secondary copper sulfide was 16.3%.	—	Yin et al. (2018)
Saishitang copper mine	Hainan Tibetan Autonomous Prefecture, Qinghai Province, China	High-altitude (3450 m); located in Qinghai–Tibet Plateau; bioleaching experiment and plant under extremely high and cold environment; total Cu 0.83%, mainly copper sulfide ore and oxide copper ores.	—	Yin et al. (2018)
Dongchuan copper mine	Dongchuan City, Yunnan Province, China	Built in 1960s, performed copper bioleaching experiments with the whole plants successful; total Cu of 0.9%–1.5%, in which 33% was sulfide ore and 41% was oxide ores.	Bornite chalcocite chalcopyrite	Yin et al. (2018)
Dongguashan copper mine	Tongling City, Anhui Province, China	Bioleaching experiments of low-grade chalcopyrite sample by <i>Ac ferrooxidans</i> and <i>Ac thiooxidans</i> . Total Cu was 0.94%–1.06%.	Chalcopyrite	Yin et al. (2018)
Dongxiang copper mine	Fuzhou City, Jiangxi Province, China	In situ bioleaching of low-grade primary chalcopyrite after underground blasting and crushing, high sulfur ores. Total Cu was 1.34%, in which chalcopyrite was 1.01%, chalcocite 0.33%; Fe was 30.05%, in which pyrite was 11.48%.	Chalcopyrite chalcocite pyrite	Yin et al. (2018)
Sarake copper mine	Wuqian, Xinjiang Autonomous Region, China	Based on experimental plant experiments, extraction rates reached 93.77% after 155 days, applied heaps bioleaching. Total Cu was 1.34%.	Chalcocite digenite chalcopyrite	Yin et al. (2018)

Continued

TABLE 7.2 Industrial cases of copper bioleaching across the globe.—cont'd

Typical mine	Location	Features	Minerals	References
Zhongwei copper mine	Ningxia Hui Autonomous Region, China	Based on experimental plant experiments, extraction rate reached 83.03% after 315 days; existing amount of CaSO_4 . Total Cu 0.32%, in which secondary copper sulfide was 59.38% and primary copper sulfide was 37.5%.	—	Yin et al. (2018)
Duobaoshan copper mine	Nenjiang County, Heilongjiang Province, China	Cu extraction rate just 15.5% after 326 days and CaSO_4 passivation obviously disturbed results. Total Cu was 0.51%, in which primary copper sulfide was 0.38%.	Chalcopyrite	Yin et al. (2018)
Daye copper mine	Daye City, Hubei Province, China	Total Cu 0.35%, in which copper sulfide was 32.3%, free oxide copper was 26.3%, and silicate copper was 22%. Biodump leaching, high-oxide, high-clay; copper extraction rate reached 83.97% after 80 days.	—	Yin et al. (2018)
Hami copper—nickel mine	Hami, Xinjiang Autonomous Region, China	Low-grade sulfide ores containing high magnesium; nickel and copper bioleaching; extraction rate: Cu 32.6% and Ni 84.6%.	Pyrrhotite nickel pyrite chalcopyrite	Yin et al. (2018)

TABLE 7.3 Methods used to control the oxidation-reduction potential (ORP) in chalcopyrite bioleaching.

	Methods	ORP versus SHE/mV	References
Addition of chemical reagents	KMnO ₄ (abiotic, 90°C)	542–892	Kametani and Aoki (1985)
		618, 798	Sandström et al. (2005)
	Nitrate (bacteria, 30°C, 45°C). Bacteria can adapt to nitrates	–	Shiers et al. (2014)
	20–30 mM nitrate (archaea, 60°C). Archaea cannot adapt to nitrates	628–658	
	Sodium sulfite (adjusting dissolved oxygen)	618	Sandström et al. (2005)
Adjustment of dissolved oxygen		578	Third et al. (2002)
		618	Gericke et al. (2010)
		575–675	Pinches, Gericke, & Van Rooyen, (2000)
Use of electrochemical reactors		598–623	Ahmadi et al. (2010)
		638–678	Ahmadi et al. (2011)
		598–628	Ahmadi et al. (2013)
		>600	Natarajan (1992)
		<698	Nakasono et al. (1997)
Adjustment of microorganisms	Adding sulfur-oxidizing bacteria	698	Hunter (2002)
		498–798	Córdoba et al. (2008)
		548–798	Bevilaqua et al. (2014)
	Using weak iron oxidizer (<i>Sulfobacillus thermosulfidooxidans</i>)	<798	Christel et al. (2018)
		<642	Qin et al. (2013)
	Using weak iron-oxidizer (<i>Sulfobacillus acidophilus</i> YTF1)	<600	Masaki et al. (2018)
Addition of sulfide minerals		203 + 29.5 log(aCu ²⁺), 284 + 47.3 log(aCu ²⁺)–15.77 log(aFe ²⁺)	Zhao, Wang, Gan et al. (2015), Zhao, Wang, Yang et al. (2015)

The optimum range of ORP for chalcopyrite bioleaching is commonly considered to be around 0.60–0.65 V (SHE) (Yi et al., 2013). Diverse methods used to control the ORP are listed in Table 7.3, which gives us a wealth of inspiration to explore more methods to control the ORP. Moreover, the regulation of ORP is not limited to biotic systems. Of course, further application to industry still requires plenty of work.

In general, the dissolution process of chalcopyrite in bioleaching highly depends on the redox potential. However, the mechanism of action is not totally clear. It is difficult to detect and quantify an intermediate species of Cu₂S precisely because it forms in trace amounts and is not stable in a bioleaching system, even though some publications claimed that Cu₂S

was detected at low redox potential in chalcopyrite bioleaching (Ahmadi et al., 2011; Gu et al., 2013; Liang et al., 2010; Liu et al., 2015; Zhu et al., 2011). The redox potential is mainly determined by the redox couples, especially Fe(III)-containing species and dissolved oxygen. In addition to Fe^{3+} , $\text{Fe}(\text{SO}_4)_2^-$, FeSO_4^+ , FeHSO_4^{2+} , and other Fe(III)-containing species that are formed owing to the coordination reactions between iron and sulfates are important oxidants (Yue et al., 2014, 2016). The dissolved oxygen mainly exists in the forms of a superoxide radical, hydrogen peroxide, hydroxyl radical, and other reactive oxygen species (Jones et al., 2011). On the one hand, the temperature, pH, ionic strength, solution composition, solution structure, and other various factors can directly affect the redox potential. On the other hand, these factors can affect the redox potential indirectly by influencing the conversion and balance of the Fe(III)-containing species and reactive oxygen species in the solution.

7.3.2 pH

It has been commonly recognized that pH is the key operating parameter for the dissolution and passivation of chalcopyrite in bioleaching. First, the dissolution of chalcopyrite in bioleaching is an acid consumption process; the oxidation of chalcopyrite by microorganism also supplies sulfuric acid. Second, microorganisms are sensitive to the pH of the solution, which influences the activity and growth of microorganisms. Most microorganisms grow or oxidize energy sources optimally between pH 1.5 and 2 (Plumb et al., 2008). Third, the pH can significantly affect the chemical reactions of a solution, especially the production of jarosite, which is regarded as an important candidate passivating species. A low pH is beneficial to preventing the formation of jarosite, but most thermophiles cannot tolerate an extremely low pH (<1.0), which indicates that jarosite precipitation cannot be totally prevented (Vilcáez et al., 2008). Liu et al. (Liu et al., 2016) proposed that chalcocite and bornite formed at pH 2.25 and 2.50 and covellite formed at pH 1.25–2.00 in chalcopyrite bioleaching. Using electrochemical tests, Warren et al. (Warren et al., 1982) found that chalcopyrite was passivated in anodic polarization. In this process, chalcopyrite was sensitive to pH under conditions of high potential but less sensitive to pH under conditions of low potential, and an increase in current occurred owing to water decomposition in the ultrapassivation zone under conditions of higher potential.

7.3.3 Temperature

An increase in temperature accelerates the chemical reaction kinetics. The optimum temperatures of various microorganisms are different. In chalcopyrite bioleaching, mesophilic microorganisms ($<40^\circ\text{C}$), moderately thermophilic microorganisms ($40\text{--}55^\circ\text{C}$), and extremely thermophilic microorganisms ($55\text{--}80^\circ\text{C}$) are commonly used (Johnson & Hallberg, 2003; Rohwerder et al., 2003; Schippers, 2007). In general, the highest dissolution kinetics of chalcopyrite can be obtained in an extremely thermophilic archaea system, followed by a moderately thermophilic bacteria system and mesophilic system (Fu et al., 2008; Gautier et al., 2008; Gericke et al., 2001). However, an increase in temperature also benefits the production of jarosite (Vilcáez et al., 2008). Viramontes-Gamboa et al. (Viramontes-Gamboa et al., 2007) studied the passivation of chalcopyrite at $25\text{--}80^\circ\text{C}$ and found that the passivation potential (E-pp) increased from 680 mV versus SHE at 25°C to 755 mV versus SHE at 80°C . In addition, the effect of acidity on E-pp was studied. The researchers found that acidity had little effect on E-pp at $25\text{--}40^\circ\text{C}$ and $60\text{--}80^\circ\text{C}$; however, at $40\text{--}60^\circ\text{C}$, E-pp increased from

680 to 755 mV versus SHE. They further proposed that the transition potential of chalcopyrite from nonpassivation to passivation was different compared with the transition potential from passivation to nonpassivation, which might explain the inconsistency of many experimental results with controlled redox potential leaching (Viramontes-Gamboa et al., 2010).

ORP, pH, and temperature often interact with each other in chalcopyrite bioleaching. Olson et al. (Olson et al., 2003) believed that bioleaching had considerable prospects for application and that some parameters could be controlled to strengthen the bioleaching of chalcopyrite, including a lower initial Fe^{3+} concentration, lower ORP, sufficient Fe^{2+} , fine grinding of ore, higher temperature, and the addition of moderately thermophilic bacteria or thermophilic Archaea. Vilcáz et al. (Vilcáz et al., 2008) suggested that Fe^{3+} was the key factor in the preliminary stage of leaching, whereas the temperature was the next key factor. In the middle stage of leaching, when the pH was low, the temperature was the main factor controlling the leaching process. With the consumption of H^+ , the ORP gradually became the key factor. Therefore, different parameters should be controlled at different stages to intensify chalcopyrite leaching. Compared with pH 0.9–2.0 at 40°C, jarosite is more readily formed at pH 2.0–2.6 at 30°C (Fu et al., 2008).

7.3.4 The others

Prior fine grinding or milling is often required to accelerate the dissolution kinetics of chalcopyrite in bioleaching. However, fine particles (<25 μm) can negatively affect the activity of cells because of attrition caused by the increased particle–particle collision (Nemati et al., 2000). The dissolution rate of chalcopyrite in bioleaching decreases with an increase in the pulp density. The adaptation of microorganisms to high pulp density and to toleration for high metal ion concentration is a challenge. A pulp density higher than 15% is detrimental to the activity and growth of thermophiles (Wang et al., 2014). Bioleaching of chalcopyrite at 20% pulp density has been conducted in the laboratory, but an adaptation strategy is required before bioleaching (Ahmadi et al., 2011; Wang et al., 2014). The transfer rate of oxygen increases with an increase in the agitation speed in bioleaching. However, a high speed of agitation increases the fluid shear rate, which has an adverse effect on the oxidation activity of microorganisms in bioleaching (Liu et al., 2007; Nemati & Harrison, 2000). Overall, it is desirable to find the optimum agitation manner and speed according to the actual bioleaching systems.

Studies showed that activated carbon could effectively accelerate the dissolution of chalcopyrite; the promoting effect was mainly attributed to galvanic interactions (Ahmadi et al., 2013; Liang et al., 2010; Nakazawa et al., 1998). Geocoat technology has been used to extract copper from chalcopyrite. This technology is achieved by coating a concentrate onto inert or low-grade support rock and subsequently subjected it to heap bioleaching. The customization of mineral compositions and highly exposed minerals surfaces can be realized by using Geocoat technology. Combined with commercial advantages such as lower cost, these technological advantages make this technology more competitive (Petersen & Dixon, 2006).

Chloride ions can reduce the passivation layers on the chalcopyrite surface and form a second redox couple of complex Cu(I) ions, resulting in high dissolution kinetics (Bevilaqua et al., 2013; Carneiro & Leão, 2007; Chang-Li et al., 2012; Kinnunen & Puhakka, 2004).

Watling et al. (Watling et al., 2016) used saline water to extract copper from chalcopyrite in bioleaching and found that the long-term adaptation of microorganisms was required.

Surfactants such as polysorbate and polyethylene glycol have been used to accelerate the dissolution process of chalcopyrite in bioleaching. The perfect surfactant for improving chalcopyrite bioleaching should have good wettability, chemical stability, and nontoxicity and be rich in oxyethylene groups. The presence of surfactants enhances chalcopyrite bioleaching mainly by improving the adsorption of microorganisms on the chalcopyrite surface and the oxidation of elemental sulfur (Duncan et al., 1964; Zhang, Niu et al., 2016; Zhang, Wei et al., 2016). Panda et al. (Panda et al., 2013) used the chemical combination of sodium and ammonium carbonate reagent to remove the surface passivation layer successfully, which was formed during meso-acidophilic bioleaching of chalcopyrite in two-step meso-acidophilic bioleaching. Glass beads (Pan et al., 2012) were also added to the bioleaching system to remove the passivation layer of jarosite on chalcopyrite by mechanical friction. Also, community structure controls (Feng et al., 2015; Ma et al., 2017) and visible light (Yang et al., 2017; Zhou et al., 2015) have been reported to accelerate the dissolution process of chalcopyrite in bioleaching.

7.4 Summary

The large-scale commercial application of chalcopyrite bioleaching has developed slowly owing to the refractory nature of chalcopyrite. Sites of industrial practice of copper bioleaching are mainly distributed in Chile, China, and Australia. Redox potential, pH, and temperature are key parameters in chalcopyrite bioleaching; they should be cooperatively controlled to accelerate chalcopyrite bioleaching. In addition, many additives can be used to enhance chalcopyrite bioleaching. Collectively, bioleaching is an alternative hydrometallurgical technology to conventional beneficiation-pyrometallurgy for processing low-grade, fine, and complex distributed ores because of the aspects discussed here. Bioleaching may also have an important role in processing solid waste and secondary resources.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (51704331) and the Young Elite Scientists Sponsorship Program by China Association for Science and Technology (CAST) (2017QNRC001).

References

- Ahmadi, A., Ranjbar, M., & Schaffie, M. (2013). Effect of activated carbon addition on the conventional and electrochemical bioleaching of chalcopyrite concentrates. *Geomicrobiology Journal*, 30(3), 237–244. <https://doi.org/10.1080/01490451.2012.665152>.
- Ahmadi, A., Schaffie, M., Manafi, Z., & Ranjbar, M. (2010). Electrochemical bioleaching of high grade chalcopyrite flotation concentrates in a stirred bioreactor. *Hydrometallurgy*, 104(1), 99–105. <https://doi.org/10.1016/j.hydromet.2010.05.001>.
- Ahmadi, A., Schaffie, M., Petersen, J., Schippers, A., & Ranjbar, M. (2011). Conventional and electrochemical bioleaching of chalcopyrite concentrates by moderately thermophilic bacteria at high pulp density. *Hydrometallurgy*, 106(1–2), 84–92. <https://doi.org/10.1016/j.hydromet.2010.12.007>.

- Akcil, A., Erust, C., Ozdemiroglu, S., Fonti, V., & Beolchini, F. (2015). A review of approaches and techniques used in aquatic contaminated sediments: Metal removal and stabilization by chemical and biotechnological processes. *Journal of Cleaner Production*, 86, 24–36. <https://doi.org/10.1016/j.jclepro.2014.08.009>.
- Anjum, F., Shahid, M., & Akcil, A. (2012). Biohydrometallurgy techniques of low grade ores: A review on black shale. *Hydrometallurgy*, 117–118, 1–12. <https://doi.org/10.1016/j.hydromet.2012.01.007>.
- Asghari, I., Mousavi, S. M., Amiri, F., & Tavassoli, S. (2013). Bioleaching of spent refinery catalysts: A review. *Journal of Industrial and Engineering Chemistry*, 19(4), 1069–1081. <https://doi.org/10.1016/j.jiec.2012.12.005>.
- Batty, J. D., & Rorke, G. V. (2006). Development and commercial demonstration of the BioCOP™ thermophile process. *Hydrometallurgy*, 83(1–4), 83–89. <https://doi.org/10.1016/j.hydromet.2006.03.049>.
- Bevilaqua, D., Lahti-Tommila, H., Garcia, O., Puhakka, J. A., & Tuovinen, O. H. (2014). Bacterial and chemical leaching of chalcopyrite concentrates as affected by the redox potential and ferric/ferrous iron ratio at 22 °C. *International Journal of Mineral Processing*, 132, 1–7. <https://doi.org/10.1016/j.minpro.2014.08.008>.
- Bevilaqua, D., Lahti, H., Suegama, P. H., Garcia, O., Benedetti, A. V., Puhakka, J. A., et al. (2013). Effect of Na-chloride on the bioleaching of a chalcopyrite concentrate in shake flasks and stirred tank bioreactors. *Hydrometallurgy*, 138, 1–13. <https://doi.org/10.1016/j.hydromet.2013.06.008>.
- Brierley, J. A. (2008). A perspective on developments in biohydrometallurgy. *Hydrometallurgy*, 94(1–4), 2–7. <https://doi.org/10.1016/j.hydromet.2008.05.014>.
- Brierley, J. A., & Brierley, C. L. (2001). Present and future commercial applications of biohydrometallurgy. *Hydrometallurgy*, 59(2–3), 233–239. [https://doi.org/10.1016/S0304-386X\(00\)00162-6](https://doi.org/10.1016/S0304-386X(00)00162-6).
- Carneiro, M. F. C., & Leão, V. A. (2007). The role of sodium chloride on surface properties of chalcopyrite leached with ferric sulphate. *Hydrometallurgy*, 87(3–4), 73–82. <https://doi.org/10.1016/j.hydromet.2007.01.005>.
- Chang-Li, L., Jin-Lan, X., Zhen-Yuan, N., Yi, Y., & Chen-Yan, M. (2012). Effect of sodium chloride on sulfur speciation of chalcopyrite bioleached by the extreme thermophile *Acidianus manzaensis*. *Bioresource Technology*, 110, 462–467. <https://doi.org/10.1016/j.biortech.2012.01.084>.
- Christel, S., Herold, M., Bellenberg, S., Buetti-Dinh, A., Hajjami, M. E., Pivkin, I. V., et al. (2018). Weak iron oxidation by *Sulfobacillus thermosulfidooxidans* maintains a favorable redox potential for chalcopyrite bioleaching. *Frontiers in Microbiology*, 9. <https://doi.org/10.3389/fmicb.2018.03059>.
- Clark, M. E., Batty, J. D., van Buuren, C. B., Dew, D. W., & Eamon, M. A. (2006). Biotechnology in minerals processing: Technological breakthroughs creating value. *Hydrometallurgy*, 83(1–4), 3–9. <https://doi.org/10.1016/j.hydromet.2006.03.046>.
- Colmer, A. R., & Hinkle, M. E. (1947). The role of microorganisms in acid mine drainage: A preliminary report. *Science*, 106(2751), 253–256. <https://doi.org/10.1126/science.106.2751.253>.
- Córdoba, E. M., Muñoz, J. A., Blázquez, M. L., González, F., & Ballester, A. (2008). Leaching of chalcopyrite with ferric ion. Part IV: The role of redox potential in the presence of mesophilic and thermophilic bacteria. *Hydrometallurgy*, 93(3–4), 106–115. <https://doi.org/10.1016/j.hydromet.2007.11.005>.
- Demergasso, C. S., Galleguillos, P. P. A., Escudero, G., L. V., Zepeda, A., V. J., Castillo, D., et al. (2005). Molecular characterization of microbial populations in a low-grade copper ore bioleaching test heap. *Hydrometallurgy*, 80(4), 241–253. <https://doi.org/10.1016/j.hydromet.2005.07.013>.
- Domic, E. M. (2007). A review of the development and current status of copper bioleaching operations in Chile: 25 Years of successful commercial implementation. In *Biomining* (pp. 81–95).
- Du Plessis, C. A., Batty, J. D., & Dew, D. W. (2007). Commercial applications of thermophile bioleaching. In *Biomining* (pp. 57–80). Springer-Verlag Berlin Heidelberg. https://doi.org/10.1007/978-3-540-34911-2_3.
- Duncan, D. W., Trussell, P. C., & Walden, C. C. (1964). Leaching of chalcopyrite with *thiobacillus ferrooxidans*: Effect of surfactants and shaking. *Applied Microbiology*, 122–126. <https://doi.org/10.1128/aem.12.2.122-126.1964>.
- Ehrlich, H. L. (2001). Past, present and future of biohydrometallurgy. *Hydrometallurgy*, 59(2–3), 127–134. [https://doi.org/10.1016/S0304-386X\(00\)00165-1](https://doi.org/10.1016/S0304-386X(00)00165-1).
- Erüst, C., Akcil, A., Gahan, C. S., Tuncuk, A., & Deveci, H. (2013). Biohydrometallurgy of secondary metal resources: A potential alternative approach for metal recovery. *Journal of Chemical Technology and Biotechnology*, 88(12), 2115–2132. <https://doi.org/10.1002/jctb.4164>.
- Feng, S., Yang, H., & Wang, W. (2015). Improved chalcopyrite bioleaching by *Acidithiobacillus* sp. via direct step-wise regulation of microbial community structure. *Bioresource Technology*, 192, 75–82. <https://doi.org/10.1016/j.biortech.2015.05.055>.

- Fu, B., Zhou, H., Zhang, R., & Qiu, G. (2008). Bioleaching of chalcopyrite by pure and mixed cultures of *Acidithiobacillus* spp. and *Leptospirillum ferriphilum*. *International Biodeterioration and Biodegradation*, 62(2), 109–115. <https://doi.org/10.1016/j.ibiod.2007.06.018>.
- Gautier, V., Escobar, B., & Vargas, T. (2008). Cooperative action of attached and planktonic cells during bioleaching of chalcopyrite with *Sulfolobus metallicus* at 70°C. *Hydrometallurgy*, 94(1–4), 121–126. <https://doi.org/10.1016/j.hydromet.2008.05.036>.
- Gericke, M., Govender, Y., & Pinches, A. (2010). Tank bioleaching of low-grade chalcopyrite concentrates using redox control. *Hydrometallurgy*, 104(3–4), 414–419. <https://doi.org/10.1016/j.hydromet.2010.02.024>.
- Gericke, M., Neale, J. W., & Van Staden, P. J. (2009). A mintek perspective of the past 25 years in minerals bioleaching. *Journal of the South African Institute of Mining and Metallurgy*, 109(10), 567–585. <http://www.saimm.co.za/Journal/v109n10p567.pdf>.
- Gericke, M., Pinches, A., & Van Rooyen, J. V. (2001). Bioleaching of a chalcopyrite concentrate using an extremely thermophilic culture. *International Journal of Mineral Processing*, 62(1–4), 243–255. [https://doi.org/10.1016/S0301-7516\(00\)00056-9](https://doi.org/10.1016/S0301-7516(00)00056-9).
- Gómez, C., Figueroa, M., Muñoz, J., Blázquez, M. L., & Ballester, A. (1996). Electrochemistry of chalcopyrite. *Hydrometallurgy*, 43(1–3), 331–344. [https://doi.org/10.1016/0304-386x\(96\)00010-2](https://doi.org/10.1016/0304-386x(96)00010-2).
- Gu, G., Hu, K., Zhang, X., Xiong, X., & Yang, H. (2013). The stepwise dissolution of chalcopyrite bioleached by *Leptospirillum ferriphilum*. *Electrochimica Acta*, 103, 50–57. <https://doi.org/10.1016/j.electacta.2013.04.051>.
- Hiroyoshi, N., Miki, H., Hirajima, T., & Tsunekawa, M. (2000). Model for ferrous-promoted chalcopyrite leaching. *Hydrometallurgy*, 57(1), 31–38. [https://doi.org/10.1016/S0304-386X\(00\)00089-X](https://doi.org/10.1016/S0304-386X(00)00089-X).
- Hoque, M. E., & Philip, O. J. (2011). Biotechnological recovery of heavy metals from secondary sources-An overview. *Materials Science and Engineering: C*, 31(2), 57–66. <https://doi.org/10.1016/j.msec.2010.09.019>.
- Hu, Q., Guo, X., Liang, Y., Hao, X., Ma, L., Yin, H., et al. (2015). Comparative metagenomics reveals microbial community differentiation in a biological heap leaching system. *Research in Microbiology*, 166(6), 525–534. <https://doi.org/10.1016/j.resmic.2015.06.005>.
- Hunter, C. (2002). *Method for the bacterially assisted heap leaching of chalcopyrite*.
- Johnson, D. B., & Hallberg, K. B. (2003). The microbiology of acidic mine waters. *Research in Microbiology*, 154(7), 466–473. [https://doi.org/10.1016/S0923-2508\(03\)00114-1](https://doi.org/10.1016/S0923-2508(03)00114-1).
- Jones, G. C., Corin, K. C., Van Hille, R. P., & Harrison, S. T. L. (2011). The generation of toxic reactive oxygen species (ROS) from mechanically activated sulphide concentrates and its effect on thermophilic bioleaching. *Minerals Engineering*, 24(Issue 11), 1198–1208. <https://doi.org/10.1016/j.mineng.2011.05.016>.
- Kametani, H., & Aoki, A. (1985). Effect of suspension potential on the oxidation rate of copper concentrate in a sulfuric acid solution. *Metallurgical Transactions B*, 16(4), 695–705. <https://doi.org/10.1007/BF02667506>.
- Kinnunen, P. H. M., & Puhakka, J. A. (2004). Chloride-promoted leaching of chalcopyrite concentrate by biologically-produced ferric sulfate. *Journal of Chemical Technology and Biotechnology*, 79(8), 830–834. <https://doi.org/10.1002/jctb.1051>.
- Liang, C. L., Xia, J. L., Zhao, X. J., Yang, Y., Gong, S. Q., Nie, Z. Y., et al. (2010). Effect of activated carbon on chalcopyrite bioleaching with extreme thermophile *Acidianus manzaensis*. *Hydrometallurgy*, 105(1–2), 179–185. <https://doi.org/10.1016/j.hydromet.2010.07.012>.
- Liu, X., Chen, B., Chen, J., Zhang, M., Wen, J., Wang, D., et al. (2016). Spatial variation of microbial community structure in the Zijinshan commercial copper heap bioleaching plant. *Minerals Engineering*, 94, 76–82. <https://doi.org/10.1016/j.mineng.2016.05.008>.
- Liu, H. C., Xia, J. L., & Nie, Z. Y. (2015). Relatedness of Cu and Fe speciation to chalcopyrite bioleaching by *Acidithiobacillus ferrooxidans*. *Hydrometallurgy*, 156, 40–46. <https://doi.org/10.1016/j.hydromet.2015.05.013>.
- Liu, H., Xia, J., Nie, Z., Ma, C., Zheng, L., Hong, C., et al. (2016). Bioleaching of chalcopyrite by *Acidianus manzaensis* under different constant pH. *Minerals Engineering*, 98, 80–89. <https://doi.org/10.1016/j.mineng.2016.07.019>.
- Liu, G., Yin, J., & Cong, W. (2007). Effect of fluid shear and particles collision on the oxidation of ferrous iron by *Acidithiobacillus ferrooxidans*. *Minerals Engineering*, 20(13), 1227–1231. <https://doi.org/10.1016/j.mineng.2007.06.002>.
- Masaki, Y., Hirajima, T., Sasaki, K., Miki, H., & Okibe, N. (2018). Microbiological redox potential control to improve the efficiency of chalcopyrite bioleaching. *Geomicrobiology Journal*, 35(8), 648–656. <https://doi.org/10.1080/01490451.2018.1443170>.

- Ma, L., Wang, X., Feng, X., Liang, Y., Xiao, Y., Hao, X., et al. (2017). Co-culture microorganisms with different initial proportions reveal the mechanism of chalcopyrite bioleaching coupling with microbial community succession. *Bioresource Technology*, 223, 121–130. <https://doi.org/10.1016/j.biortech.2016.10.056>.
- Nakasono, S., Matsumoto, N., & Saiki, H. (1997). Electrochemical cultivation of *Thiobacillus ferrooxidans* by potential control. *Bioelectrochemistry and Bioenergetics*, 43(1), 61–66. [https://doi.org/10.1016/S0302-4598\(97\)00001-9](https://doi.org/10.1016/S0302-4598(97)00001-9).
- Nakazawa, H., Fujisawa, H., & Sato, H. (1998). Effect of activated carbon on the bioleaching of chalcopyrite concentrate. *International Journal of Mineral Processing*, 55(2), 87–94. [https://doi.org/10.1016/S0301-7516\(98\)00026-X](https://doi.org/10.1016/S0301-7516(98)00026-X).
- Natarajan, K. A. (1992). Bioleaching of sulphides under applied potentials. *Hydrometallurgy*, 29(1–3), 161–172. [https://doi.org/10.1016/0304-386X\(92\)90011-N](https://doi.org/10.1016/0304-386X(92)90011-N).
- Needham, J., Ling, W., Ping-yü, H., & Lu, G. (1976). *Science and civilisation in China: Chemistry and chemical technology. Part 3. Spagyric discovery and invention: Historical survey, from cinnabar elixirs to synthetic insulin*.
- Nemati, M., & Harrison, S. T. L. (2000). Effect of solid loading on thermophilic bioleaching of sulfide minerals. *Journal of Chemical Technology and Biotechnology*, 75(7), 526–532. [https://doi.org/10.1002/1097-4660\(200007\)75:7<526::AID-JCTB249>3.0.CO;2-4](https://doi.org/10.1002/1097-4660(200007)75:7<526::AID-JCTB249>3.0.CO;2-4).
- Nemati, M., Lowenadler, J., & Harrison, S. T. L. (2000). Particle size effects in bioleaching of pyrite by acidophilic thermophile *Sulfolobus metallicus* (BC). *Applied Microbiology and Biotechnology*, 53(2), 173–179. <https://doi.org/10.1007/s002530050005>.
- Olson, G. J., Brierley, J. A., & Brierley, C. L. (2003). Bioleaching review part B: Progress in bioleaching: Applications of microbial processes by the minerals industries. *Applied Microbiology and Biotechnology*, 63(3), 249–257. <https://doi.org/10.1007/s00253-003-1404-6>.
- Panda, S., Parhi, P. K., Nayak, B. D., Pradhan, N., Mohapatra, U. B., & Sukla, L. B. (2013). Two step meso-acidophilic bioleaching of chalcopyrite containing ball mill spillage and removal of the surface passivation layer. *Bioresource Technology*, 130, 332–338. <https://doi.org/10.1016/j.biortech.2012.12.071>.
- Panda, S., Sanjay, K., Sukla, L. B., Pradhan, N., Subbaiah, T., Mishra, B. K., et al. (2012). Insights into heap bioleaching of low grade chalcopyrite ores – a pilot scale study. *Hydrometallurgy*, 125–126, 157–165. <https://doi.org/10.1016/j.hydromet.2012.06.006>.
- Pant, D., Joshi, D., Upreti, M. K., & Kotnala, R. K. (2012). Chemical and biological extraction of metals present in E waste: A hybrid technology. *Waste Management*, 32(5), 979–990. <https://doi.org/10.1016/j.wasman.2011.12.002>.
- Pan, H. D., Yang, H. Y., Tong, L. L., Zhong, C. B., & Zhao, Y. S. (2012). Control method of chalcopyrite passivation in bioleaching. *Transactions of Nonferrous Metals Society of China*, 22(9), 2255–2260. [https://doi.org/10.1016/S1003-6326\(11\)61457-X](https://doi.org/10.1016/S1003-6326(11)61457-X).
- Pathak, A., Dastidar, M. G., & Sreekrishnan, T. R. (2009). Bioleaching of heavy metals from sewage sludge: A review. *Journal of Environmental Management*, 90(8), 2343–2353. <https://doi.org/10.1016/j.jenvman.2008.11.005>.
- Petersen, J., & Dixon, D. G. (2006). Competitive bioleaching of pyrite and chalcopyrite. *Hydrometallurgy*, 83(1–4), 40–49. <https://doi.org/10.1016/j.hydromet.2006.03.036>.
- Pinches, A., Gericke, M., & Van Rooyen, J. (2000). A method of operating a bioleach process with control of redox potential. *Patent WO*.
- Plumb, J. J., Muddle, R., & Franzmann, P. D. (2008). Effect of pH on rates of iron and sulfur oxidation by bioleaching organisms. *Minerals Engineering*, 21(1), 76–82. <https://doi.org/10.1016/j.mineng.2007.08.018>.
- Qin, W., Yang, C., Lai, S., Wang, J., Liu, K., & Zhang, B. (2013). Bioleaching of chalcopyrite by moderately thermophilic microorganisms. *Bioresource Technology*, 129, 200–208. <https://doi.org/10.1016/j.biortech.2012.11.050>.
- Qin, W., Zhen, S., Yan, Z., Campbell, M., Wang, J., Liu, K., et al. (2009). Heap bioleaching of a low-grade nickel-bearing sulfide ore containing high levels of magnesium as olivine, chlorite and antigorite. *Hydrometallurgy*, 98(1–2), 58–65. <https://doi.org/10.1016/j.hydromet.2009.03.017>.
- Reza, M. A., Sadrnezhaad, K. S., & Zahra, M. (2014). Simulation of bioleaching heat effects for enhancement of copper recovery from Sarcheshmeh chalcopyrite. *Metallurgical and Materials Transactions B*, 1204–1212. <https://doi.org/10.1007/s11663-014-0036-9>.
- Rohwerder, T., Gehrke, T., Kinzler, K., & Sand, W. (2003). *Bioleaching Review Part A: Applied Microbiology and Biotechnology*, 239–248. <https://doi.org/10.1007/s00253-003-1448-7>.
- Rossi. (1990). *Biohydrometallurgy*. Hamburg: McGraw-Hill.
- Ruan, R., Liu, X., Zou, G., Chen, J., Wen, J., & Wang, D. (2011). Industrial practice of a distinct bioleaching system operated at low pH, high ferric concentration, elevated temperature and low redox potential for secondary copper sulfide. *Hydrometallurgy*, 108(1–2), 130–135. <https://doi.org/10.1016/j.hydromet.2011.03.008>.

- Ruan, R., Wen, J., & Chen, J. (2006). Bacterial heap-leaching: Practice in Zijinshan copper mine. *Hydrometallurgy*, 77–82. <https://doi.org/10.1016/j.hydromet.2006.03.048>.
- Ruan, R., Zhong, S., & Wang, D. (2010). Life cycle assessment of two copper metallurgical processes: Bio-heapleach and flotation-flash smelter. *China Nonferrous Metallurgy*, 39(4), 30–33.
- Sandström, A., Shchukarev, A., & Paul, J. (2005). XPS characterisation of chalcopyrite chemically and bio-leached at high and low redox potential. *Minerals Engineering*, 18(5), 505–515. <https://doi.org/10.1016/j.mineng.2004.08.004>.
- Schippers, A. (2007). Microorganisms involved in bioleaching and nucleic acid-based molecular methods for their identification and quantification. In *Microbial processing of metal sulfides* (pp. 3–33). Springer Netherlands. https://doi.org/10.1007/1-4020-5589-7_1.
- Shiers, D. W., Ralph, D. E., & Watling, H. R. (2014). The effects of nitrate on substrate utilisation by some iron(II)- and sulfur-oxidising bacteria and archaea. *Hydrometallurgy*, 150, 259–268. <https://doi.org/10.1016/j.hydromet.2014.07.009>.
- Soto, P. E., Galleguillos, P. A., Serón, M. A., Zepeda, V. J., Demergasso, C. S., & Pinilla, C. (2013). Parameters influencing the microbial oxidation activity in the industrial bioleaching heap at Escondida mine, Chile. *Hydrometallurgy*, 133, 51–57. <https://doi.org/10.1016/j.hydromet.2012.11.011>.
- Third, K. A., Cord-Ruwisch, R., & Watling, H. R. (2002). Control of the redox potential by oxygen limitation improves bacterial leaching of chalcopyrite. *Biotechnology and Bioengineering*, 78(4), 433–441. <https://doi.org/10.1002/bit.10184>.
- Vilcáez, J., & Inoue, C. (2009). Mathematical modeling of thermophilic bioleaching of chalcopyrite. *Minerals Engineering*, 22(11), 951–960. <https://doi.org/10.1016/j.mineng.2009.03.001>.
- Vilcáez, J., Suto, K., & Inoue, C. (2008). Bioleaching of chalcopyrite with thermophiles: Temperature-pH-ORP dependence. *International Journal of Mineral Processing*, 88(1–2), 37–44. <https://doi.org/10.1016/j.minpro.2008.06.002>.
- Vilcáez, J., Yamada, R., & Inoue, C. (2009). Effect of pH reduction and ferric ion addition on the leaching of chalcopyrite at thermophilic temperatures. *Hydrometallurgy*, 96(1–2), 62–71. <https://doi.org/10.1016/j.hydromet.2008.08.003>.
- Viramontes-Gamboa, G., Peña-Gomar, M. M., & Dixon, D. G. (2010). Electrochemical hysteresis and bistability in chalcopyrite passivation. *Hydrometallurgy*, 105(1–2), 140–147. <https://doi.org/10.1016/j.hydromet.2010.08.012>.
- Viramontes-Gamboa, G., Rivera-Vasquez, B. F., & Dixon, D. G. (2007). The active-passive behavior of chalcopyrite comparative study between electrochemical and leaching responses. *Journal of the Electrochemical Society*, 154(6).
- Wang, Y., Zeng, W., Qiu, G., Chen, X., & Zhou, H. (2014). A moderately thermophilic mixed microbial culture for bioleaching of chalcopyrite concentrate at high pulp density. *Applied and Environmental Microbiology*, 80(2), 741–750. <https://doi.org/10.1128/AEM.02907-13>.
- Warren, G. W., Wadsworth, M. E., & El-Raghy, S. M. (1982). Passive and transpassive anodic behavior of chalcopyrite in acid solutions. *Metallurgical Transactions B*, 13(4), 571–579. <https://doi.org/10.1007/BF02650014>.
- Watling, H. R. (2008). The bioleaching of nickel-copper sulfides. *Hydrometallurgy*, 91(1–4), 70–88. <https://doi.org/10.1016/j.hydromet.2007.11.012>.
- Watling, H. R., Collinson, D. M., Corbett, M. K., Shiers, D. W., Kaksonen, A. H., & Watkin, E. L. J. (2016). Saline-water bioleaching of chalcopyrite with thermophilic, iron(II)- and sulfur-oxidizing microorganisms. *Research in Microbiology*, 167(7), 546–554. <https://doi.org/10.1016/j.resmic.2016.05.003>.
- Wu, A., Yin, S., Wang, H., Qin, W., & Qiu, G. (2009). Technological assessment of a mining-waste dump at the Dexing copper mine, China, for possible conversion to an in situ bioleaching operation. *Bioresource Technology*, 100(6), 1931–1936. <https://doi.org/10.1016/j.biortech.2008.10.021>.
- Xiao, Y., Liu, X., Liang, Y., Niu, J., Zhang, X., Ma, L., et al. (2016). Insights into functional genes and taxonomical/phylogenetic diversity of microbial communities in biological heap leaching system and their correlation with functions. *Applied Microbiology and Biotechnology*, 100(22), 9745–9756. <https://doi.org/10.1007/s00253-016-7819-7>.
- Yang, B., Gan, M., Luo, W., Zhou, S., Lei, P., Zeng, J., et al. (2017). Synergistic catalytic effects of visible light and graphene on bioleaching of chalcopyrite. *RSC Advances*, 7(79), 49838–49848. <https://doi.org/10.1039/c7ra10015a>.
- Yin, S., Wang, L., Kabwe, E., Chen, X., Yan, R., An, K., et al. (2018). Copper bioleaching in China: Review and prospect. *Minerals*, 8(2). <https://doi.org/10.3390/min8020032>.
- Yi, Y., Weihua, L., & Miao, C. (2013). A copper and iron K-edge XANES study on chalcopyrite leached by mesophiles and moderate thermophiles. *Minerals Engineering*, 31–35. <https://doi.org/10.1016/j.mineng.2013.01.010>.

- Yue, G., Guezennec, A. G., & Asselin, E. (2016). Extended validation of an expression to predict ORP and iron chemistry: Application to complex solutions generated during the acidic leaching or bioleaching of printed circuit boards. *Hydrometallurgy*, 164, 334–342. <https://doi.org/10.1016/j.hydromet.2016.06.027>.
- Yue, G., Zhao, L., Olvera, O. G., & Asselin, E. (2014). Speciation of the $\text{H}_2\text{SO}_4\text{-Fe}_2(\text{SO}_4)_3\text{-FeSO}_4\text{-H}_2\text{O}$ system and development of an expression to predict the redox potential of the $\text{Fe}^{3+}/\text{Fe}^{2+}$ couple up to 150°C. *Hydrometallurgy*, 147–148, 196–209. <https://doi.org/10.1016/j.hydromet.2014.05.008>.
- Zhang, X., Niu, J., Liang, Y., Liu, X., & Yin, H. (2016). Metagenome-scale analysis yields insights into the structure and function of microbial communities in a copper bioleaching heap. *BMC Genetics*, 17(1). <https://doi.org/10.1186/s12863-016-0330-4>.
- Zhang, R., Wei, D., Shen, Y., Liu, W., Lu, T., & Han, C. (2016). Catalytic effect of polyethylene glycol on sulfur oxidation in chalcopyrite bioleaching by *Acidithiobacillus ferrooxidans*. *Minerals Engineering*, 95, 74–78. <https://doi.org/10.1016/j.mineng.2016.06.021>.
- Zhao, H., Wang, J., Gan, X., Zheng, X., Tao, L., Hu, M., et al. (2015). Effects of pyrite and bornite on bioleaching of two different types of chalcopyrite in the presence of *Leptospirillum ferriphilum*. *Bioresource Technology*, 194, 28–35. <https://doi.org/10.1016/j.biortech.2015.07.003>.
- Zhao, H., Wang, J., Tao, L., Cao, P., Yang, C., Qin, W., et al. (2017). Roles of oxidants and reductants in bioleaching system of chalcopyrite at normal atmospheric pressure and 45°C. *International Journal of Mineral Processing*, 162, 81–91. <https://doi.org/10.1016/j.minpro.2017.04.002>.
- Zhao, H., Wang, J., Yang, C., Hu, M., Gan, X., Tao, L., et al. (2015). Effect of redox potential on bioleaching of chalcopyrite by moderately thermophilic bacteria: An emphasis on solution compositions. *Hydrometallurgy*, 151, 141–150. <https://doi.org/10.1016/j.hydromet.2014.11.009>.
- Zhou, S., Gan, M., Zhu, J., Li, Q., Jie, S., Yang, B., et al. (2015). Catalytic effect of light illumination on bioleaching of chalcopyrite. *Bioresource Technology*, 182, 345–352. <https://doi.org/10.1016/j.biortech.2015.02.010>.
- Zhu, W., Xia, J.L., Yang, Y., Nie, Z.y., Zheng, L., Ma, C.y., et al. (2011). Sulfur oxidation activities of pure and mixed thermophiles and sulfur speciation in bioleaching of chalcopyrite. *Bioresource Technology*, 102(4), 3877–3882. <https://doi.org/10.1016/j.biortech.2010.11.090>.

This page intentionally left blank

Index

Note: 'Page numbers followed by "f" indicate figures and "t" indicate tables.'

A

a-chalcopyrite, 147–148
Acid mine drainage (AMD), 3, 125
Acidianus
 A. ambivalens, 13–14
 A. tengchongensis, 13–14
Acidihalobacter prosperus, 10–11
Acidiphilium, 6–7
Acidithiobacillia, 7, 10–11, 14–15
Acidithiobacillus spp., 1–3, 6–7
 A. caldus, 1–2, 25, 27–28, 30, 128–129, 202–203
 A. ferrovorans, 1–2, 6–7, 32–33
 A. ferrooxidans, 1–2, 6–7, 28, 112
 copper dissolution with, 114
 A. thiooxidans, 13–15, 30–33, 31t–32t
 Acidithiobacillus-like populations, 13–14
 characteristics, 32–33
 metabolic diversity, 33–40
Acidophiles, 2
Acidophilic microorganisms, 14
Acidophilic prokaryotes, 2, 10–11
Adenosine triphosphate (ADP), 9–10
Adenylsulfate reductase (APS reductase), 13–14
Adsorption energy, 187–188
Ag recovery from bioleaching residue, 169–170
Ammonium, 10
Ammonium monooxygenase, 10
Anisotropic crystal surface, 149
Arthrobacter sp., 15
Atmospheric nitrogen, 10
ATP citrate lyase (ACL), 9–10
Autotrophic carbon fixation, 9–10
Average nucleotide identity (ANI), 15
Average nucleotide identity based on BLAST (ANiB), 15

B

b-chalcopyrite, 147–148
Band theory, 110–111
Biohydrometallurgy, 2, 211
Bioleaching
 behavior, 135–140, 157–158

 of chalcopyrite, 73–74, 94–95
 flowsheets, 211–212
 recovery of Cu in, 202–203
 technology, 211–214
Bornite, 177
 effect of, 177
 reduction of, 76
Brillouin zone integrations, 187

C

Calvin cycle, 34
Calvin–Benson–Bassham cycle (CBB cycle), 9
Carbohydrate esterases (CEs), 25–26
Carbohydrate-active enzymes (CAZymes), 25–26
Carbon assimilation, 34–37
Carbon paste electroactive electrode, 164–165
Carbonates, 177
Catalytic mechanism of silver ions in chalcopyrite
 bioleaching, 183–200. *See also* Passivation
 mechanism of chalcopyrite bioleaching
 density functional theory calculations on catalytic
 mechanism of Ag⁺, 187–192
 dissolution experiments, 184–187
 surface properties, 196–199
 surface species, 192–196
Catalyzed bioleaching using silver-containing waste,
 201–205. *See also* Bioleaching
 comprehensive process, 201
 recovery of
 Ag in thiourea leaching, 203–205
 Cu in bioleaching, 202–203
Caudovirales, 6–7
Cell exudates, 26
Central carbon metabolism, 21–27
Chalcocite (Cu₂S), 77, 108–110, 214
Chalcopyrite (CuFeS₂), 51, 157
 bioleaching, 214, 225
 industrial practice cases, 214
 key parameters and controlling techniques, 214–226
 industrial cases of copper bioleaching across
 globe, 215t–222t
 methods used to control ORP, 223t

- Chalcopyrite (CuFeS_2) (*Continued*)
 others, 225–226
 pH, 224
 redox potential/oxidation-reduction potential, 214–224
 temperature, 224–225
 leaching, 98
 passive film, 74
 properties of
 (0 0 1) surface, 60–64
 (1 1 2) surface, 64–68
 electronic structure, 57–59
 oxidation state of Cu, Fe, and S elements, 51–57
 reconstruction of chalcopyrite surfaces, 60–68
 technology, 211–214
 comparative techno-economic analysis, 213t
 flowsheet of conventional beneficiation-pyrometallurgy and, 213f
 schematic diagram of bioleaching of copper ores, 212f
- Charge transfer resistance, 198–199
- Chemical phase analysis, 203–205
- Chloride ions (Cu(I) ions), 225–226
- Citryl-CoA lyase, 9–10
- Citryl-CoA synthetase, 9–10
- Coding sequences (CDS), 4
- Conventional beneficiation-pyrometallurgy, 211–212
- Copper (Cu)
 copper-bearing sulfide ore, 118
 oxidation state of Cu elements, 51–57
 recovery from bioleaching residue, 169–170
- Core genes, 19–20
- Corrosion current density (I_{corr}), 198
- Corrosion potential, 166–168
- Covellite, 53–54
- Critical redox potential, 109
- Cupric ions, 109, 117
- Cyclic voltammetry (CV), 74
- Cytochrome c (Cyc2), 11–13
- D**
de novo assembly, 4
- Density functional theory (DFT), 57–58
 calculations on catalytic mechanism of Ag^+ , 187–192
- Dicarboxylate-4-hydroxybutyrate cycle, 9
- Dissolution experiments, 184–187
- Dissolution mechanism
 mineralogical properties, 135–149
 bioleaching behavior, 135–140
 electrochemical analysis, 143–144
 intermediate species analysis, 140–142
- Dissolution process for chalcopyrite, 98
- Disulfide, 73–74, 194–195
 passivation of chalcopyrite surface, 94–98
- Doping, 147
- E**
 E-pp, 224–225
- Electro-bioreactor, 98
- Electrochemical analysis, 143–144
- Electrochemical behavior of chalcopyrite, 73–87
- Electrochemical dissolution process of chalcopyrite
 disulfide and polysulfide passivation of chalcopyrite surface, 94–98
 electrochemical behavior of chalcopyrite, 73–87
 enhancing bioleaching of chalcopyrite by controlling solution potential, 112–118
 optimum range of redox potential for chalcopyrite leaching, 98–112
 surface species of chalcopyrite after treatment by different potentials, 87–93
- Electrochemical impedance spectroscopy (EIS), 73, 133, 196–197
- Electrochemistry analysis, 164–169
- Electron correlation effects, 57–58
- Electronic structure of chalcopyrite, 57–59
- Elemental sulfur (S^0), 73–74, 91–93, 106, 126–127
- Euryarchaeota*, 7
- Ex situ spectroscopic methods, 73
- Exchange and correlation potential (XC), 57–58
- F**
 Faradaic admittance
 of chalcopyrite oxidation or passive film formation, 82
 of passive film oxidation, 82
- Ferric ions, 76, 98, 116–117
- Ferric iron (Fe(III)), 2
- Ferric sulfates, 127–128
- Ferroplasma* sp., 128–129
 F. acidophilum, 3
- Ferrous ions, 109, 114
- Ferrous iron (Fe(II)), 2
 oxidation, 10–13
- Flexible genes, 19–20
- fox* operon, 10–11
- Full width at half maximum (FWHM), 53, 161–163
- Functional annotation, 4
- G**
 g-chalcopyrite, 147–148
- Galvanic effect, 171–173
- Galvanox, 157
- Gangue minerals in chalcopyrite bioleaching, 157, 177–178
 marmatite in chalcopyrite bioleaching, 157–170

pyrite in chalcopyrite bioleaching, 170–176
 Gene prediction, 4
 Generalized gradient approximation (GGA), 57–58
 Gibbs free energy, 109
 Glutamine, 10
 Glycolic acid, 26
 Glycoside hydrolases (GHs), 25–26
 Guanine plus cytosine (GC), 15–17
 Guanosine diphosphate (GDP), 24–25

H

Hall effect, 135–137
 Heterodisulfide reductase (*hdrABC*), 13–14
 Homologous genes, 1–2
 Hydroxyl radicals, 2
 Hydroxylamine oxidoreductase, 10
 3-hydroxypropionate bicycle, 9, 21–24
 3-hydroxypropionate/4-hydroxybutyrate cycle, 9

I

in situ electrochemical techniques, 73
 Indirect mechanism model, 184
 Inductively coupled plasma-atomic emission spectrometry (ICP-AES), 99, 202–203
 Insoluble sulfate (SO_4^{2-}), 126–128
 Intermediate species analysis, 130–133, 140–142, 158–164

J

Jarosite, 73–74, 101, 106, 127–128, 203–205

K

KAAS online server, 20
 Key parameters, 214–226

L

Leptospirillum, 3, 6–7
L. ferriphilum, 14–15, 21–25, 28, 184
L. ferrodiazotrophum, 14–15
L. ferrooxidans, 10–11
L. rubrum, 14–15

Local density of states (LDOS), 61
 Low critical redox potential (E_L), 110
 Lysates, 26

M

Marmatite in chalcopyrite bioleaching, 157–170
 bioleaching behavior, 157–158
 Cu and Ag recovery from bioleaching residue, 169–170
 electrochemistry analysis, 164–169
 intermediate species analysis, 158–164
 Metabolic function, 32–33

Metagenome datasets, taxonomic assignment of, 4–7, 5t
 Metagenomic DNA, 4
 Metagenomics, 3–4
 Metal-deficient sulfide, 73–74
 Metallic oxides, 195–196
 Metallic silver, 203–205
 Microbial communities, structure composition and functional diversity of, 3–14
 Mineralogical properties on passivation and dissolution mechanism, 135–149
 Mixed potential, 99–100
 Monosulfide, 194–195
 Mulliken population of chalcopyrite, 59

N

nifD gene, 10
nifE gene, 10
nifH gene, 10
nifK gene, 10
nifN gene, 10
nifX gene, 10
 Nitrate, 10
 Nitrite, 10
 Nitrogen
 metabolism, 10
 uptake, 27–28

O

Open circuit potential (OCP), 74, 133, 171, 196–197
 massive chalcopyrite electrodes, 75f
 value of, 197–198
 Optimum range of redox potential for chalcopyrite leaching, 98–112
 effect of Cu^{2+} ions on chalcopyrite leaching, 103–107
 impact of electronic structure on leaching of chalcopyrite, 110–112
 Fe^{2+} accelerating dissolution of chalcopyrite, 99–102
 thermodynamic calculation, 108–110
 Oxidation state of Cu, Fe, and S elements, 51–57
 Oxidation-reduction potential (ORP), 128–129, 133, 157–158, 184, 214–224
 in chalcopyrite bioleaching, 223t
 mediating, 174–176
 Oxidation-reduction reactions, 98
 Oxidative corrosion potential (E_{corr}), 198
 Oxide ores, 177

P

Passivation layer resistance, 199
 Passivation mechanism of chalcopyrite bioleaching, 133–135. *See also* Dissolution mechanism
 bioleaching behavior, 128–130

Passivation mechanism of chalcopyrite bioleaching
(Continued)

- intermediate species analysis, 130–133
- mineralogical properties, 135–149
 - bioleaching behavior, 135–140
 - electrochemical analysis, 143–144
 - intermediate species analysis, 140–142
- passivating species, 125–128
- pH, 224
- Phase, 147–148
- pio* operon, 10–11
- Polyethylene glycol, 226
- Polysorbate glycol, 226
- Polysulfide (S_n^{2-}), 73–74, 126–127, 194–195
 - passivation of chalcopyrite surface, 94–98
- Potentiodynamic polarization of chalcopyrite, 79
- Potentiostatic polarization experiments, 144
- Potentiostatic polarization of chalcopyrite, 85–86
- Proton motive force (PMF), 29
- Pyrite (FeS_2), 56–57
 - in chalcopyrite bioleaching, 170–176
 - galvanic effect, 171–173
 - mediating oxidation-reduction potential, 174–176

Q

Quartz mineral, 177–178

R

- Reaction surface, 194–195
- Redox potential, 98, 106, 214–224
- Reduced inorganic sulfur compounds (RISCs), 13
- Reduction mechanism, 174–176
- Reductive acetyl-coenzyme A pathway (acetyl-CoA pathway), 9
- Reductive tricarboxylic acid cycle (rTCA cycle), 9, 21–24
- Rhodanobacter*, 6–7
- Rhodobacter capsulatus*, 10–11
- Rhodospseudomonas palustris*, 10–11
- Ribosomal RNA (rRNA), 3–4
- Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), 9
- Rusticyanin genes (*rus*), 11–13

S

- Scanning electron microscope (SEM), 202–203
- Sedoheptulose-1,7-bisphosphatase (SBPase), 9
- Self-consistent field (SCF), 57–58
- Semiconductor property, 145–147
- Sequencing, 4
- Silicates, 177
- Silver chloride ($AgCl$), 203–205
- Silver ions (Ag^+ ions), 183

- catalytic mechanism of silver ions in chalcopyrite bioleaching, 183–200
- catalyzed bioleaching using silver-containing waste, 201–205

- Silver oxide (Ag_2O), 203–205
- Silver sulfate (Ag_2SO_4), 203–205
- Silver sulfide (Ag_2S), 203–205
- Silver-bearing solid waste, 201
- Species biodiversity, 1–2
- Sphalerite unit cell, 51, 52f
- Sphingopyxis* sp., 15
- Stepwise bioleaching, 158–161
- Sterile leaching of chalcopyrite, 137–140
- Sulfate adenylyltransferase (SAT), 13–14
- Sulfide ores, 177
- Sulfide quinone reductase (SQR), 13
- Sulfobacillus* spp., 14–15
 - S. benefaciens*, 14–15
 - S. sibiricus*, 14–15
 - S. thermosulfidooxidans*, 1–2, 14–15, 25–27, 30, 112, 128–129
 - S. thermotolerans*, 14–15
- Sulfur oxygenase reductase (SOR), 13–14
- Summed Gaussian–Lorentzian function (SGL function), 130–131
- Surface reconstruction, 55
- Surface species, 192–196
 - of chalcopyrite after treatment by different potentials, 87–93
- Sweeping potential, 77
- Synchrotron radiation-based X-ray diffraction (SR-XRD), 186–187

T

- Tafel test, 198
- Ternary chalcopyrite semiconductors, 147
- Tetranucleotide frequency correlation coefficient (TETRA), 15
- Tetrathionate, 13–14
- Tetrathionate hydrolase (TetH), 13
- Thermodynamic calculation, 108–110
- Thiobacillus*, 6–7
 - T. prosperus*, 10–11
- Thiosulfate, 127–128
- Thiosulfate sulfurtransferase (*tst*), 13–14
- Thiosulfate:quinone oxidoreductase (TQO), 13
- Thiourea, 170
 - recovery of Ag in thiourea leaching, 203–205

U

- Uridine diphosphate (UDP), 24–25
 - UDP-galactose, 24–25
 - UDP-glucose, 24–25

UDP-glucuronate, 24–25

UDP-N-acetyl-glucosamine, 24–25

V

Vienna ab-initio simulation package (VASP), 187

X

X-ray diffraction analysis (XRD), 128–129

X-ray photoelectron spectroscopy (XPS),
52–53, 52f, 58f, 74, 87, 127–128,
158–161

XC. *See* Exchange and correlation potential (XC)

Z

Zinc ferrite (ZnFe_2O_4), 201

Zinc leaching residue (ZLR), 157, 201

This page intentionally left blank

BIOHYDROMETALLURGY OF CHALCOPYRITE

Explains the mechanism of chalcopyrite bioleaching and discusses various leaching processes.

Hongbo Zhao

Central South University, Changsha, Hunan, China

Congren Yang

Central South University, Changsha, Hunan, China

Xian Zhang

Central South University, Changsha, Hunan, China

Yisheng Zhang

Central South University, Changsha, Hunan, China

Guanzhou Qiu

Central South University, Changsha, Hunan, China

Bioleaching of chalcopyrite is a challenging and popular topic of research. The low copper extraction and dissolution kinetics has restricted the industrial application of chalcopyrite bioleaching. To solve this problem, first the dissolution process and passivation mechanism of chalcopyrite in bioleaching should be studied. Then, the rate-limiting steps should be explicitly analyzed, and finally, an intensifying method can be put forward. Many scholars have attempted to investigate the dissolution mechanism of chalcopyrite in bioleaching. However, no agreement has yet been reached.

Biohydrometallurgy of Chalcopyrite summarizes and discusses the reported research findings. In addition, this book publishes related results found by the authors' research. Then, the dissolution mechanism of chalcopyrite in bioleaching is interpreted. Finally, the process intensification techniques of chalcopyrite bioleaching are provided and discussed. This book provides a useful reference and guidance for both laboratory research and industrial production.

Key Features

- Interprets the dissolution mechanism of chalcopyrite in bioleaching
- Provides feasible technologies for intensifying chalcopyrite bioleaching
- Offers an overview of chalcopyrite bioleaching
- Gives readers a deep understanding of the bioleaching mechanisms of chalcopyrite
- Provides topics for future research and potential industrial applications



ELSEVIER

elsevier.com/books-and-journals

ISBN 978-0-12-821880-8



9 780128 218808