



Presentation on mechanisms and applications of chalcopyrite and pyrite bioleaching in biohydrometallurgy – a presentation



Huang Tao ^{a,b,*}, Li Dongwei ^{a,b}

^a College of Resource and Environmental Science, Chongqing University, Chongqing 400044, China

^b State Key Laboratory for Coal Mine Disaster Dynamics and Control, Chongqing University, Chongqing 400044, China

ARTICLE INFO

Article history:

Received 26 February 2014

Received in revised form 20 August 2014

Accepted 10 September 2014

Available online 16 September 2014

Keywords:

Structural characteristics

Bioleaching mechanisms

Applications

Chalcopyrite

Pyrite

Biohydrometallurgy

ABSTRACT

This review outlines classic and current research, scientific documents and research achievements in bioleaching, particularly in respect of the bioleaching of chalcopyrite and pyrite. The diversity and commonality of the microbial leaching process can be easily studied through comparing the bioleaching mechanism and the application of these two metal sulfides. The crystal, electronic and surface structures of chalcopyrite and pyrite are summarized in detail in this paper. It determines the specific and complicated interaction pathways, kinetics of the atmospheric/aqueous oxidation, and the control process of bioleaching of the minerals as the precondition. Bioleaching of metal sulfides is performed by a diverse group of microorganisms and microbial communities. The species of the bacteria which have a significant effect on leaching ores are miraculously diverse. The newly identified acidophilic microorganisms with unique characteristics for efficient bioleaching of sulfidic minerals are increasing sharply.

The cell-to-cell communication mechanisms, which are still implicit, elusive and intangible at present day, have gradually become a research hotspot. The different mineralogy characteristics and the acid solubility of the metal sulfides (e.g., chalcopyrite and pyrite) cause two different dissolution pathways, the thiosulfate and the polysulfide pathways. The bioleaching mechanisms are categorized by contact (an electrostatic attachment) and noncontact (planktonic) process, with emphasis on the produce of extracellular polymeric substances and formation of biofilm on the surface of the metal sulfides in this paper. The division of the direct and indirect effect are not adopted due to the redox chain, the reduction of the ferric iron and oxidation of the ferrous iron. The molecular oxygen is reduced by the electrons extracted from the specific metal sulfide, via a redox chain forming a supercomplex spanning the periplasmic space and connecting both outer and inner membrane. The passivation of the mineral surface can obviously hinder the dissolution of metal sulfides during the bioleaching process, which is significantly affected by the kinetic model, microenvironment on the surface of ore and the leach conditions, such as temperature, pH and E_h .

The new development of mechanism research, enhanced and intensified technologies on the bioleaching of chalcopyrite and pyrite, are conducted and summarized from the different branches of natural science. Some are depicted and explained based on molecular level in this paper. Catalyst and catalytic mechanisms in bioleaching and biooxidation for this two sulfide minerals have been concluded and applied for several decades, the continuous emergence of the new material and technology are also gradually applied into the biohydrometallurgy. The industrial applications of the bioleaching on chalcopyrite and pyrite are totally based on the understanding of the interaction mechanism between microbes and minerals, the optimization of ore leaching conditions and the development of new material and the leaching equipment.

It is not incredible and unimaginable to take a different bioleaching process and diagram to deal with the two sulfuric metals, which is vital to succeed in elevating the leaching rate of copper.

© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/3.0/>).

* Corresponding author at: Chongqing University, College of Resource and Environmental Science, Chongqing 400044, China. Tel.: +86 18875039043.

Contents

1. Introduction	108
2. Chalcopyrite and pyrite structures	109
2.1. Crystal structure	109
2.2. Electronic structure	109
2.3. Surface structure	110
3. Bioleaching mechanisms	111
3.1. Microbial diversity	111
3.2. The thiosulfate pathway and the polysulfide pathway	111
3.2.1. The thiosulfate pathway (pyrite)	111
3.2.2. The polysulfide pathway (chalcopyrite)	112
3.3. Attached and planktonic effect	112
3.4. Extracellular polymeric substances, biofilm formation and passivation	114
4. Catalysis and galvanic interaction	114
5. Applications of biohydrometallurgy	115
5.1. Bioleaching and biooxidation technology	116
5.2. Chloride application in the bioleaching process	116
Acknowledgements	117
References	117

1. Introduction

It is widely accepted that, in many cases, the heavy metals wrapped in complex sulphide ores are difficult, not-environment-friendly and costly to be leached with conventional mineral processing methods [1]. With the depletion of the easy-to-process ores, the energy costs and the growing movement toward sustainable mining are increasing. The practices of biohydrometallurgy are gradually accepted in the commercial applications. The low production costs and relatively small environmental pollution that makes biohydrometallurgy been efficiently used to process low-grade copper minerals and refractory ores [2–4]. The technology and technique of the bioleaching, oxidation and complexation processes, which are supported and promoted by the developments in the fields of hydrometallurgy, geology, microbiology, chemical analysis, mineralogy, surface science and molecular biology. These have been applied and employed widely for the recovery of the heavy metals from sulfuric minerals and ores, such as copper, nickel, zinc, cobalt and uranium [4–7].

Operation and applications of biohydrometallurgy in industries are artificially divided into two terms, bioleaching and bio-oxidation. The first term is related to the solubilization of base metals such as copper, nickel, and zinc from the ores, whereas biooxidation is used for the bioleached solubilized metals which are wrapped, or locked, in sulfide minerals, in most cases, iron and arsenic, and some precious metal, typically gold and silver [8]. Recently, the advantages and superiority in industrial processes through the usage and deployment of thermophiles, moderate thermophile and extreme thermophile have been demonstrated. It has effectively avoided the issues and problems that are quite common in processes using psychrophilic and mesophilic bacteria, such as cooling of leaching system, acid mine/rock drainage and some other environmental problems [9,10]. Accurately, there are two bioleaching modes, contact and non-contact leaching modes, which is now gradually accepted instead of the classified modes of direct mechanism and indirect mechanism [11,12]. The exist evidences of the direct enzymatic oxidation for the sulfur part of heavy metal sulfides cannot be demonstrated and testified. Non-contact leaching is basically exerted by planktonic bacteria, which oxidize ferrous ions in solution. While the contact leaching takes into account that most of ores dissolution is through the medium of the extracellular polymeric substances (EPS) in the specific microenvironment [13]. It should be clear that the analysis of bacterial–mineral interfaces at the molecular scale and potential

mechanism of cell to cell communication systems are still unknown or fragmented [14,15]. Bacterial attachment to the surface of the sulphidic minerals is determined by two branches, the biochemical properties of the bacteria and the interfacial properties of the minerals in a bioleaching system [16]. Most bacteria tend to be attracted to mineral surfaces by chemotaxis [17], and bacterial cells often gather in or focus on the crystal boundaries of ores [18].

Recently, biohydrometallurgical extraction of copper from low-grade chalcopyrite ore, which is quite abundant and widespread in the earth's crust, especially applied to heap bioleaching, is paid more attention. Many studies and researches have been done for that, while the operation is yet to be applied successfully at industrial and commercial scale, due to the extremely slow leach kinetics and low leaching rate. The problem that causes the delay of the application is commonly attributed to the passivation on the surface of chalcopyrite [19–21]. Sulfur, jarosite, disulfide and polysulfide are gotten and identified in the biofilm, while there is no generally accepted theory that can wholly explain the mechanism of biofilm formation [3,22–24]. Pyrite (FeS_2) is the most abundant metal sulfide associated with the earth's surface region, which is commonly considered as 'Fool's gold'. Pyrite is frequently found in massive hydrothermal deposits, sedimentary beds, veins and replacements, and igneous rock, its reserve is ample and luxuriant, to some extent [25]. Rickard and Luther have estimated that, there is about 5 million tons of pyrite being produced annually in the oceanic environment, simply due to the biogenic reduction of aqueous sulfate [26]. Pyrite is often associated with valuable minerals such as sphalerite, chalcopyrite and galena, and pyrite is commonly used for production of sulfuric acid during the process of leaching [27]. Galvanox™ is frequently referred when the combination of the chalcopyrite and pyrite is used in the leach pulp (slurry) [28,29]. The name Galvanox™ is given due to the galvanic interaction between chalcopyrite and pyrite in ferric sulfate media [30]. Heaps and stirred tanks are two different commercial and engineering applications in terms of biohydrometallurgy of sulfide minerals, based on the mechanisms of bioleaching and mineral biooxidation, which have been purposefully amended and accurately improved from the traditional metallurgical craft since the mid-1980s. Currently heap leaching accounts approximately for 20% of the worldwide copper production [31] and an estimated about one fifth of the world's copper produced from run-of-mine and crushed ores through bioleaching heap can be reached. In the process of the heap

bioleaching and mineral biooxidation, the ores and minerals, which are pretreated by metallurgical and mining methods and stacked on waterproof layers (polymer materials) are continually irrigated with the mixture of a dilute sulfuric acid solution and acidophilic microorganisms. After a period of time leaching solution that contains the released and enriched metal, are collected at the bottom and transported to the upstream of the traditional metallurgical sections or plants. Compared with heap bioleaching, requirements for industrial applications of tank bioleaching are more strict and specific, and the operating cost and capital of the systematic processes is also accordingly higher. The stirred-tank reactors appear to be usually used in the continuous flow mode of operation and often reserved for high-value metals with substantial leaching rate more than that of heap bioleaching [32,33].

2. Chalcopyrite and pyrite structures

2.1. Crystal structure

The information of the crystal structures on some common minerals can be easily gotten through an database platform, named Crystallography Open Database (COD), which is an open-access collection of crystal structures of organic, inorganic, metal-organic compounds and minerals [34]. The information of the crystal structures on chalcopyrite and pyrite are listed as followed (Tables 1 and 2):

Chalcopyrite pertains to one of the I-III-VI₂ type semiconductors with tetrahedral coordination and S atoms are displaced slightly toward the Fe atoms with a certain direction deviation. Cu is located at the fractional coordinates of (0,0,0) and (0,0.5,0.25), S is at (0.2575,0.25,0.125) and Fe is at (0,0,0.5) and (0,0.5,0.75), that the former location of Fe has spin α compared with the latter has spin β , which gives the character of antiferromagnetic structure to chalcopyrite at room/indoor temperature., and some variation in these values has listed as, $d_{\text{Fe-S}} = 2.26 \text{ \AA}$, $d_{\text{Cu-S}} = 2.30 \text{ \AA}$ and $d_{\text{Cu-Fe}} = d_{\text{Cu-Cu}} = d_{\text{Fe-Fe}} = 3.71 \text{ \AA}$ [35–38]. Pyrite is one of two polymorphic forms. FeS₂ has a face-centered crystal, which is more stable and steady than marcasite. The unit cell of pyrite is totally determined by cell parameter a , and coefficient of S, u . The crystal structure of pyrite was published in 1914 by Bragg, and the parameters that now commonly accepted are listed as $a = 5.416 \text{ \AA}$ and $u = 0.385 \text{ \AA}$. S atoms are connected by covalent bond, and share Fe²⁺ with the same five S in a slightly deformed octahedral cell. The cubic pyrite morphology which is most common in the nature, possesses the surface 100 while pyritohedral and octahedral morphologies is with surfaces 210 and 111, respectively and surface 110 are also can be found. All of these surfaces are of lower coordination as

Table 1
Parameters of chalcopyrite crystal structure.

Chemical name	Copper iron sulfide
Mineral name	Chalcopyrite
Formula	–Cu Fe S ^{2–}
a	5.24 Å
b	5.24 Å
c	10.3 Å
α	90°
β	90°
γ	90°
Cell volume	282.8 Å ³
Number of distinct elements	3
Hermann-mauguin symmetry space group	I-4 2 d
Has coordinates	Yes
Has disorder	No
Has F_{obs}	No

Table 2
Parameters of pyrite crystal structure.

Chemical name	Iron sulfide (1/2)
Mineral name	Pyrite
Formula	–Fe S ^{2–}
a	5.41 ± 0.005 Å
b	5.41 Å
c	5.41 Å
α	90°
β	90°
γ	90°
Cell volume	158.3 Å ³
Number of distinct elements	2
Has coordinates	No
Has disorder	No
Has F_{obs}	No

compared to the bulk structure as bonds are fractured during cleavage [39,40]. Usually, the cell of crystal structure of pyrite is a cube, while the structure cell of a dodecahedron with pentagonal faces or octahedral crystals with triangular faces also can be detected under a certain and specific geological tectonic environment. Specific elements can be found in the pyrite lattice as substitutions or occluded as inclusions, and the natural pyrite shows p -type or n -type conductivity in terms of the characters of semiconducting mineral [27,41,42].

2.2. Electronic structure

The valence band structure of chalcopyrite has been studied from different aspects for many years. Analysis of documents from XPS explicitly indicate the exist of Cu(I) and S^{2–} and data of Fe 2p spectra of lab and synchrotron XPS on chalcopyrite demonstrates the evidence of the bond Fe³⁺–S^{2–} [27]. Fujisawa et al. studied the electronic structures of CuFeS₂ and CuAl_{0.9}Fe_{0.1}S₂ by observing the phenomenon and analyzing the data of the states of Fe and Cu, and the valence-band of unit cell. The S 3p-Fe 3d bonding is found covalent base on the obvious tail of the XPS spectra of Cu 2p and S 2p [43]. Mikhlin et al. compared and analyzed the abraded chalcopyrite and bornite in a vacuum chamber by X-ray absorption near-edge structure (XALES) to exam the electronic structure [44]. The result showed the Cu L_{3} -edge had a strong pre-edge peak and a small post-edge peak, the Fe $L_{2,3}$ -edge energy was consistent with the Fe²⁺ oxidation state and S L -edge spectra was clearly observed [44]. It is widely accepted that the Neel temperature of CuFeS₂ is extremely high, at 823 K [45,46]. Edelbro et al. proposed that the energy bands (–13.8 to 12.5 eV), which is lower than Fermi level, is similar to that of sphalerite. Woolley et al. demonstrated that, at temperature above 50K and in an unit cell of CuFeS₂, the spin orientation of face-centered Cu is same with Cu around the face-centered Fe and is opposite with the Fe in the square (face-centered and peripheral) and Cu that is out of the square, the same situation applies to Fe [46,47]. Petiau et al. presented that the Fermi level is greater than the top of the valence-band (Cu 3d) by 0.15 eV and lower than the bottom of the conduction-band (Fe 3d) by 0.3 eV in terms of energy, based on the record of XAS measurements and analysis of band structures [48]. The energy gap between the valence-band and the conduction-band is 0.45 eV, which is consistent with the observations of other band gap. Pearce et al. combined 2p XPS and L -edge XAS with Mössbauer data to study the states of Fe and Cu, which identified the presence of high-spin Fe³⁺ in chalcopyrite [49,50]. de Oliveira and Duarte employed the density functional theory to study the magnetic structure of chalcopyrite and found the presence of Cu⁺ and Fe³⁺ [51,52].

It can be calculated that the shortest distance between atom in an unit cell of pyrite crystal is $d_{\text{S-S}} = 2.20 \text{ \AA}$ or $d_{\text{S-S}} = 2.14 \text{ \AA}$, which

appears between two anion pairs, the others length is listed as, $d_{\text{Fe-S}} = 2.26 \text{ \AA}$ and $d_{\text{Fe-S}} = 2.27 \text{ \AA}$ and there is no evidence to test the exist of S–S covalence bond [42,53,54]. Folmer et al. and van der Heide et al. constructed a model on a molecular orbital (MO) diagram of the S_2^{2-} anion, displaying the phenomenon of the orbital overlap and orbital hybridization (3s and 3p) of S atoms, based on the Mössbauer studies and XPS measurements [53]. Subsequently, Edelbro et al. proposed a band structure of FeS_2 , which is systematic and complete, calculated by using a full potential density functional approach, to some extent, similar to the calculations made by Philpott et al. [42,54]. Surface redox processes are initiated by first quenching of high energy dangling bonds and leading to the formation of new surface species. Rosso et al. revealed the surface electronic heterogeneity of UHV fractured surfaces by using STM microscopy and spectroscopy together with LEED, UPS [55,56]. Qiu et al. suggested that the transfer of reactions tend to be much faster during the process of oxidation reactions, resulted from the reduced band energy gap and intensified metallic characters and the probabilities of occurrence to Fe is much larger than S due to the bond cleavage [57]. Nesbitt et al. reached a set of values of valence band spectra on fractured pyrite surface, in the vacuum by using the synchrotron XPS. Seven peaks were identified from 0.8 eV to 16 eV, two peaks were identified in the doublet-like region, at 16 and 13 eV respectively, resulted from the function of S 3s orbital, and sp^3 hybridization of S molecular orbital cannot be demonstrated by any data [58,59]. The bond lengths of the S–S and Fe–S is tended to be shortened due to the higher dangling bond density [57]. It is presented that the tendency of spin polarization of low coordination sites is quite common compared with spin neutral of the sites and the paramagnetic class is more inclined to react with the sites with low coordination defects.

2.3. Surface structure

Synchrotron XPS is quite known for the suitability to the study of fractured and oxidized surfaces of chalcopyrite with the characters of greater surface sensitivity and spatial and spectral resolution [60,61]. Harmer et al. detected $2\text{p}_{3/2}$ spectra of S on a fresh fractured surface by synchrotron XPS to reached the main symmetric peak (161.33 eV), which is caused by the fully-coordinated bulk S atoms. Another peak at 161.88 eV is analyzed by the surface S_n^{2-} and a value (160.84 eV) is explained by the presence of surface S^{2-} . The chalcopyrite surfaces 001, 012, 100, 101, 110, 111 and 112 and surfaces of reconstructions have all been studied [51,52,62,63]. Klauber proposed that the S_2^{2-} detected on fresh fractured surfaces of chalcopyrite through simultaneous reconstruction of surfaces (mechanical) and redox process (bio-chemical), could form a pyrite-like surface layer [64]. de Oliveira and Duarte represented that ferric ions (Fe^{3+}) on the surface are normally reduced to ferrous ions (Fe^{2+}), Cu ions are likely to be oxidized and the S ions is either oxidized or reduced based on the specific leaching conditions due to characters of the valence and conduction bands [52]. Von Oertzen et al. represented that there are same amount of metal ions and S ions (atoms) on the surface 012 and the metal ions and S ions (atoms) are obviously divided in the relative position respectively on the surface 112 [62,63]. The exist of conchoidal surface on chalcopyrite is quite common, that usually caused by poor cleavages in the ore and some cationic and anionic dangling bonds (M^{n+} , S^2 , S_2^{2-} , S_2^{2-}) are contained on a fractured surface [58]. Liu et al. suggested that the substitution of cations or anions can more probably occur in the crystal cell of *p*-type chalcopyrite than the *n*-type one [65].

Surface structure and character of pyrite have been carried out from different aspects with mineral powder, fractured surfaces, as-grown surfaces and those associated with synthetic thin films.

More recent studies have used synchrotron-based PES to further suggest that there are at least two chemically identifiable sulfur monomer species. The poor cleavage and fractured conchoidal form are mostly being observed on the plane 100 and they also can be found on the surfaces 021, 111 and 110 [66]. Pettenkofer et al. presented that there are at least three factors that obviously influence the form of the S 2p region, a bulk S_2^{2-} at 162.7 eV, a surface shifted S_2^{2-} at 162 eV and a portion at 161.2 eV which is associated with the surface defect of FeS_2 , through probing the 100 cleavage plane of natural pyrite (FeS_2) by photoelectric scan (PES) with synchrotron radiation (200 eV) [67]. Bronold et al., from the direction of ligand-field theory, proposed that the valence band edge of the surface states is controlled by the lower coordination number of surface-Fe [68]. Nesbitt et al. prudentially revised the seminal model and suggested that the cleavage and crack can cause the generation of a fresh surface and also can result in the rupture of S–S bands on under certain conditions [69]. Leiro et al., suggested that scission feature of S 2p could be attributed to monomeric sulfur at kink sites that exist between the surface 100 terraces on the conchoidally fractured surface through investigating a pyrite cube [70]. To interpret and understand the operational mechanism of S 2p and Fe 2p PES, the quantum mechanical computational techniques are also widely used by many researchers and detailed experimental conclusion can be gotten by referring to their articles [62]. More recent studies have used synchrotron-based PES to further suggest that there are at least two chemically identifiable sulfur monomer species. There are many unit cell structure, which are formed by different plane [71]. The natural pyrite commonly contains a wide band of trace elements and some common metal, metalloid and non-metallic elements. Abraitis et al. summarized the mechanism and phenomenon of impurities occurred in natural pyrite [4,72]. According the data of unit cell, crystal structure and shape parameters and data of electronic and surface structures, the simulated models of chalcopyrite and pyrite are followed as Figs. 1 and 2.

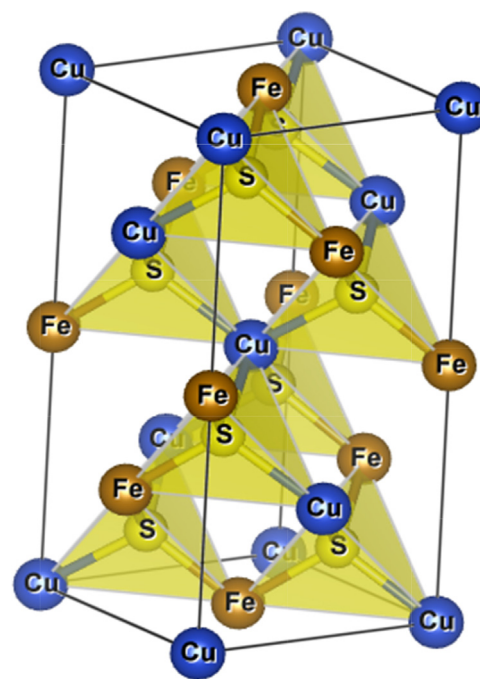


Fig. 1. Model of chalcopyrite unit cell structure. Simulation of the chalcopyrite unit cell structure, S-yellow spheres, Cu-blue spheres, Fe-brown spheres. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

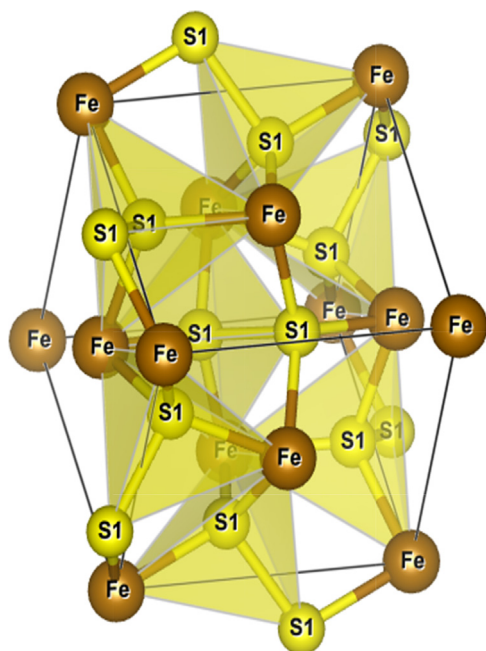


Fig. 2. Model of pyrite unit cell structure. Simulation of the pyrite unit cell structure, S-yellow spheres, Fe-brown spheres. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Bioleaching mechanisms

3.1. Microbial diversity

There are usually a series of features and characters in common shared by bioleaching microorganism or microbes. They are able to catalyze regeneration of ferric iron from ferrous iron and protons from sulfur species, grow autotrophically by fixing CO_2 from the atmosphere and adapt to low pH, high concentration of metal ions and moderate nutritional requirement [73]. It is the strong demand of the commercial system on the identification and the metabolic mechanism of microorganisms that make the genera and species of microorganisms which are suitable and adaptable for the bioleaching of metal sulfide ores or deposits be continuously found and researched. The microbial bioleaching communities which is commonly consisted by a vast variety of microorganisms in mining system, complex microbial interactions and nutrient patterns are still yet systematically understood and mastered [74,75]. In spite of the accelerated development of biohydrometallurgy, there are only a modest number of iron(II)- and sulfur oxidizing bacteria have been isolated from metal sulfide ores, described systematically and phylogenetically [76,77]. There are several reviews that afford the comprehensive and relatively complete descriptions of the mesophilic, moderately thermophilic, extremely thermophilic bacteria and archaea involved in biohydrometallurgy, and there are several recent reviews that conclude the microbial diversity related to the bioleaching and biooxidation in detail [9,10,21,78–80]. In terms of the ferrous- and sulfur-oxidizing chemolithotrophic microorganism, the acidophilic bacteria and archaea are preferred in biohydrometallurgy [79]. These acidophilic bacteria and archaea widely distributed and adapted well. They can be cultured and isolated from environments such as hot springs, volcanic regions and acid mine drainage [74,75]. The techniques such as denaturing gradient gel electrophoresis (DGGE), 16S rRNA sequencing, PCR-based methods and fluorescence in situ hybridization (FISH) are used for the identification of the

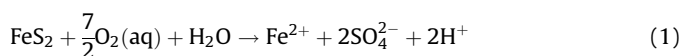
specific microorganism. Mesophilic and moderately thermophilic microorganisms spanned four bacteriophyla, the *Proteobacteria*, *Nitrospirae* and *Actinobacteria* and the extremely thermophilic archaea mostly classified to the *Sulfolobales* [8,81]. Pradhan et al. provided the listing of the autotrophic and heterotrophic bacteria and archaea that can be utilized.

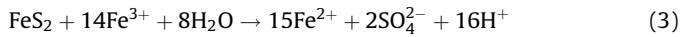
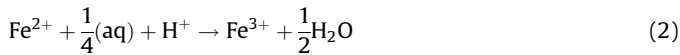
3.2. The thiosulfate pathway and the polysulfide pathway

Silverman and Ehrlich proposed that bacteria or microorganisms oxidize metal sulfide ores or deposits by a direct mechanism or an indirect mechanism. According to the different electronic extraction processes, the process that the electrons are directly transferred to the cell attached to the mineral surface from the metal sulfide is called direct bioleaching. The process that the electrons are transmitted to the oxidizing agent of the sulphide ores, ferric ions, is called indirect bioleaching. Tributsch proposed that the term “contact” leaching be used in place of “direct” leaching based on the attachment and planktonic phenomenon of the bacteria in the process of leaching [82]. Rawlings suggested that the process of the dissolution of metal sulfide and intermediates by planktonic bacteria should be described as “cooperative leaching” [12]. Crundwell summarized that the microorganisms catalyze the oxidation of ferrous ions to ferric ions, in the process of both indirect leaching and indirect contact leaching and proposed that the microorganisms interact with the metal sulfide mineral by following three mechanisms [83]. Bacteria oxidize ferrous ions to ferric ions in the bulk solution, and the ferric ions oxidize the sulfur moiety. Bacteria attached to the mineral surface oxidize ferrous ions to ferric ions within a biofilm comprised of bacteria and extracellular polymeric material (EPS), and the ferric ions generated within this layer oxidize the sulfur. Bacteria attached to the surface of the mineral oxidize the sulfur directly, without any requirement for ferric or ferrous ions is considered as the direct contact mechanism. While the evidence and signals of a direct electron transport through catalyzing by enzymes and some other organelles of the cell, between the metal sulfide and the attached cell has not been found up to now. The terms, contact leaching and non-contact leaching have been proposed for bioleaching by attached and planktonic cells, respectively. The oxidation of the acid-insoluble metal sulfide (e.g., pyrite, tungstenite, molybdenite,) and acid soluble metal sulfide (e.g., chalcopyrite, pyrrhotite, and sphalerite) can be categorized into two pathways, the thiosulfate intermediate pathway and polysulfide intermediate pathway [11,84].

3.2.1. The thiosulfate pathway (pyrite)

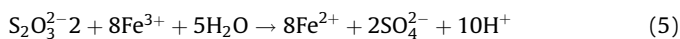
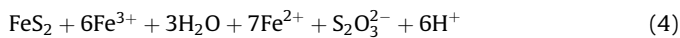
Pyrite (FeS_2) is composed of a ferrous (Fe^{2+}) ion and S_2^{-2} ion with the Fe/S ratio of 1:2. Deviations (<1%) from this stoichiometric relationship have been densely reported [72]. Pyrite oxidation is essentially important in flotation and leaching mineral ores or deposits [85] and biogeochemical cycling of Fe ions and S ions in the ecology of Fe- and S-oxidizing bacteria [86] through the production of sulfuric acid as a result of oxidation [87]. Oxidation of pyrite surfaces may occur upon exposure to atmospheric O_2 and water [85] and the oxidized layer can hinder against further oxidation and further control the subsequent processes on aqueous phase oxidation [88]. Singer et al. described the aqueous oxidation of pyrite with stoichiometric chemical reactions and the Eqs. (1), (2) and (3) are listed as followed [89],



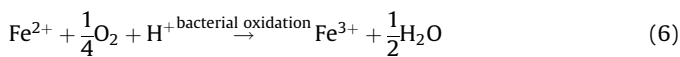


O_2 molecule and Fe^{3+} ions have been recognized as the two most important oxidants for pyrite oxidation. Moses et al. proposed that oxidation rates of pyrite in the saturated Fe^{3+} solution were two orders of magnitude higher than that due to dissolved oxygen (DO) at the condition of low pH [86,90]. The sulfur of pyrite is oxidized to the soluble sulfur intermediates after the initial attack of the oxidizing agent, ferric (Fe^{3+}). The bonds between S_2^{2-} and Fe^{2+} are cleaved, and hydrated ferrous iron ions and thiosulfate [91,92] are formed, then the soluble thiosulfate intermediate is oxidized to tetrathionate [93]. Whereafter the tetrathionate is decomposed and degraded into elemental sulfur, sulfite, trithionate and pentathionate [87,92,94]. At the end, these sulfur compounds are completely oxidized to sulfate in the solution, the schematic diagram is showed as followed (Fig. 3)

The related equations are listed as the followed:



Balci et al. proposed that the dominant bacterial role is likely to oxidize the ferrous ions to ferric ions which catalyzes the followed Reaction (6) because ferric is still the main oxidizing agent [95,96].



3.2.2. The polysulfide pathway (chalcopyrite)

The leaching rate of chalcopyrite (CuFeS_2) is known to be quite slow and tends to be depressed with time [97,98], that is the main resistance and obstruction to the commercial application. The metal-sulfur bonds can be cleaved by the assault or attack of the protons, which analyze its acid-solubility and quite different from the pyrite. It has been widely studied that the sulfur moiety of these metal sulfides is oxidized mostly into elemental sulfur at low pH condition [99]. Carneiro et al. detected elemental sulfur on the surface of the chalcopyrite in the solution of ferric sulfate (FeSO_4)

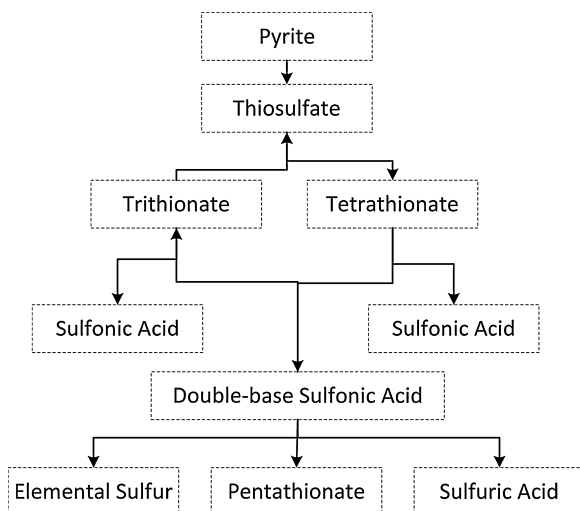
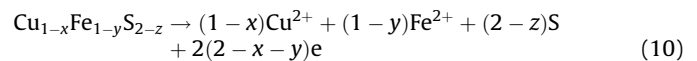
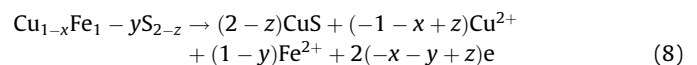
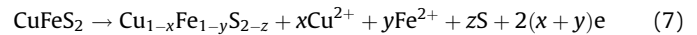
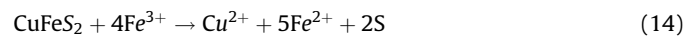
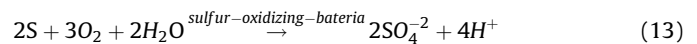
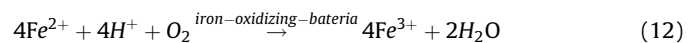
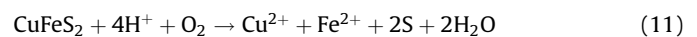


Fig. 3. Intermediates of the thiosulfate pathway on pyrite.

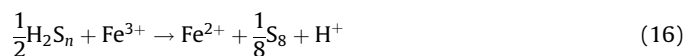
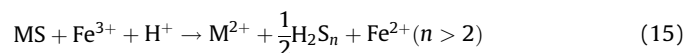
or ferric chloride (FeCl_3) under the conditions of low-temperature [100,101], which is considered to be related with the obstinate character on dissolution. There are a battery of chemical and biochemical reactions that explain the formation of elemental sulfur through the polysulfide pathway on the surface of acid-soluble metal sulfide minerals. The metal-deficient sulfides ($\text{Cu}_{1-x}\text{Fe}_{1-y}\text{S}_{2-z}$) are intermediate product phases of chalcopyrite dissolution in acidic and oxidizing solution, at the condition of low temperatures [4,102–104] or at high temperatures [105]. Warren et al. presented the formation of bornite, which is considered as a passive intermediate product phase in acidic sulfate solutions based on the thermodynamic analysis [106]. The equations are listed as followed:



The leaching chemical mechanism of solubilization of chalcopyrite has been shown as followed equations. The bioleaching and biooxidation of the minerals are functioned by the microbes or archaea that responsible for producing ferric iron and sulfuric acid used for the leaching of copper [107].



The general mechanism on polysulfide pathway can be explained by the following equations and the leaching mechanism of the thiosulfate pathway and the polysulfide pathway are showed as Fig. 4 [13,108].



3.3. Attached and planktonic effect

As aforementioned, the bioleaching mechanisms can be categorized through contact, un-contact and cooperative mechanisms. The attachment and contact of the bacteria are mediated by secretion of extracellular polymeric substance (EPS) surrounding the bacteria [17,109,110]. It is found that more than 80% bacteria of

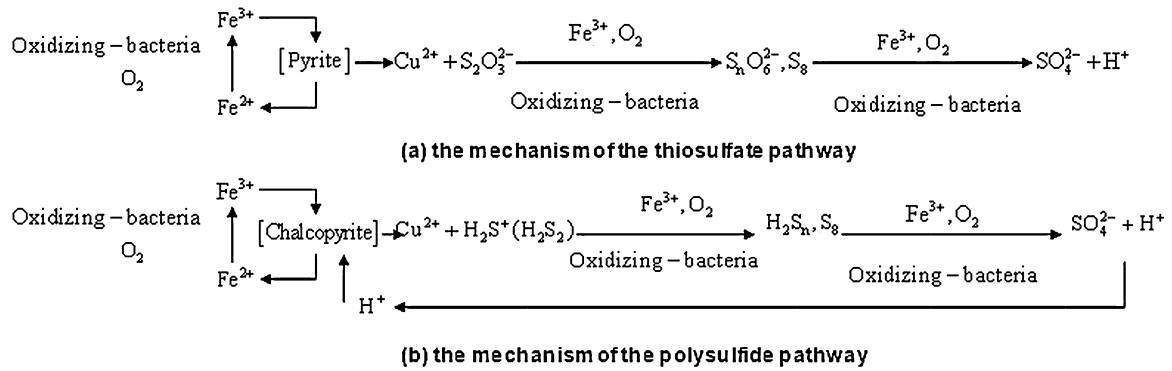
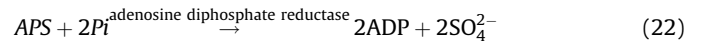
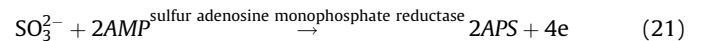


Fig. 4. The schematic diagram of the leaching mechanism of the thiosulfate pathway and the polysulfide pathway.

an inoculum can disappear from the solution a day later on an infinite surface space [111]. In detail, Rodriguez et al. presented that contact process can be divided into three stages, the process of extensive bacterial attachment, a decrease in bacterial attachment due to surface saturation and cooperation between contacted and planktonic microorganism [17]. Attachment or surface contact stimulates the production of EPS [112,113]. The bacteria attached to the mineral surface oxidize ferrous ions in the solution to ferric ions by the enzymatic catalyst to extract electrons from the mineral surface. It reduces molecular oxygen within bacterial membranes through a complex redox chain. Blake et al. found the electric properties of the bacteria and pyrite surface were obviously different. The positively charged cells mostly attached to the negatively charged pyrite surface, at pH 2 in sulfuric acid solution due to the electrostatic interactions [114,115]. The attachment of the bacteria to the sulphide surfaces are somewhat influenced by hydrophobic interactions, especially in terms of the hydrophobic surfaces. It can be frequently observed that the preferred sites on the surface of metal sulfide are in or around the cracks and defects of the surface [116]. Meyer et al. verified the tropotaxes or chemotaxis of the bacteria by detecting that *At. ferrooxidans* and *L. ferrooxidans* reacted actively to gradients of ferrous ions, ferric ions, thiosulfate, etc. [117]. Rimstidt and Vaughan summarized the mechanisms and chained phenomenon of the chemotaxis of the bacteria from the aspect of the electrochemical direction, presented the anodes and cathodes are formed by the chemotaxis of the bacteria on the surface of the pyrite that has imperfections in the crystal lattice where the iron-to-sulfur ratio is not exactly 1/2 [118]. The cooperative mechanism is used to describe the interactions between the attached and planktonic bacteria. The contacted microorganism transfer substrate to breed the planktonic ones through the EPS surrounding them and the planktonic bacteria supply oxidants to enhance the leaching efficiency [119]. Singer et al. found that there are two cytochromes in *L. ferrooxidans* that are essentially related to the ferrous oxidation in the aerobic condition, Cyt₅₇₂ and Cyt₅₇₉ [120]. Blake and Griff detected that the redox status of the solution that is full of cellular cytochrome would obviously control the iron respiratory chain of *L. ferrooxidans* in the aerobic condition [114]. There are two pathway, “downhill” or an “uphill” pathway, can be used for the transportation of electrons extracted from the ferrous ions. It is widely accepted that the rus operon encodes the proteins that involved in the “downhill” pathway. Rus is frequently considered as a vital constituent part of the iron respiratory chain in *At. ferrooxidans* with oxygen as electron acceptor at pH 2 which treated as an electron reservoir in the transfer process of electrons [121,122]. The differences in ATP levels between attached and planktonic cells of *Acidithiobacillus ferrooxidans* growing with elemental sulfur, the cellular ATP content was 1.01amol per attached cell and 0.34amol per planktonic cell, which was

attributed to sulfur limitation in the planktonic cells. S⁰ is oxidized by the S-oxidizing bacteria through a complex system. S⁰ is imported into the membrane through the cytoderm and is combined by glutathione (GSH), forming a kind of activated polysulfide, which is finally oxidized into sulfate or sulfuric acid by the function of sulfur oxidase, sulfur adenosine monophosphate reductase and adenosine diphosphate reductase, the equations are listed as followed,



The process of the attached and planktonic effect of the iron(II)- and S-oxidizing bacteria and transfer of electrons in *At. ferrooxidans* is graphed as Figs. 5 and 6.

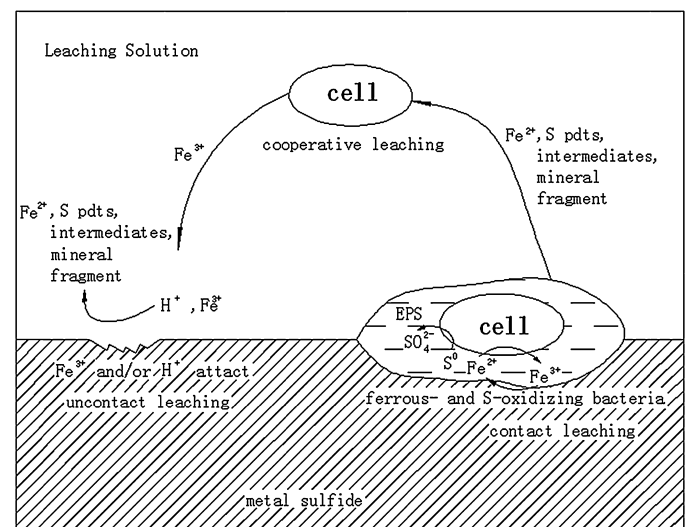


Fig. 5. The contact, un-contact and cooperative leaching mechanisms of the bacteria.

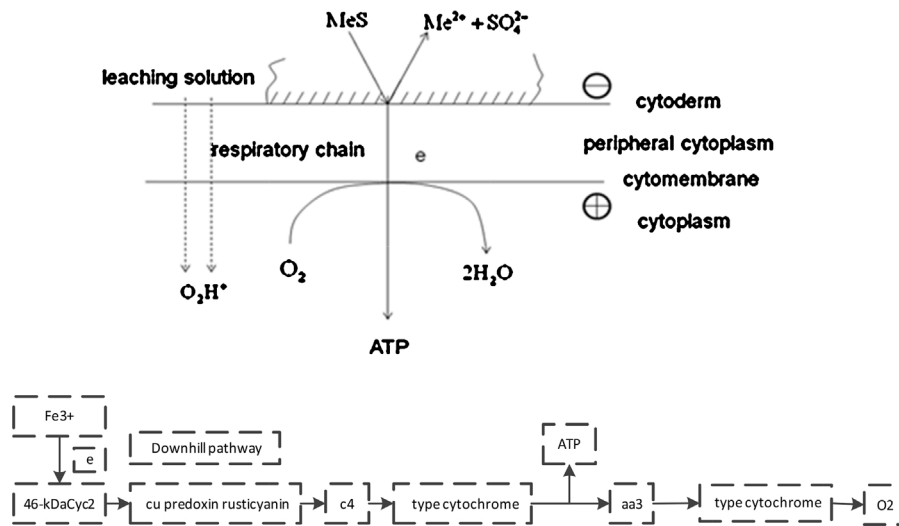
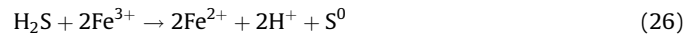
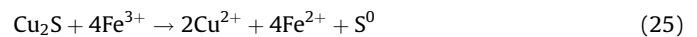
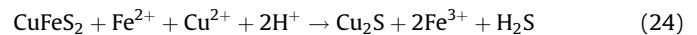


Fig. 6. The downhill pathway of transfer of electrons in *At. ferrooxidans*.

3.4. Extracellular polymeric substances, biofilm formation and passivation

The components of EPS of different ferrous- and S-oxidizing bacteria coupling with different leaching conditions have been widely studied. Gehrke et al. verified that the EPS of *At. ferrooxidans* consists of the sugars glucose, rhamnose, fucose, xylose, mannose, C12–C20 saturated fatty acids, glucuronic acid, and ferric ions, on the surface of pyrite [123,124]. The compositions and amount of components of EPS would change when the bacteria adapted to the new substrate in the solution. Sharma et al. found the surface charges were different between the bacteria grown in the solution with ferrous ions and those dwell at the surface of the metal sulfide or sulfur due to the difference of protein content [125]. Arredondo et al. demonstrated that the attachment functionality of the bacteria was assisted and enhanced by lipopolysaccharides and some specific cell surface proteins [126]. The ferric ions was combined by uronic acids through complexation in EPS, which facilitated the biooxidation. Cells grown on the surface of elemental sulfur do not effectively attach to the surface of FeS₂ due to a potentially changed EPS composition compared with that of the pyrite-grown cells. Pronk et al. showed that, in aerobic conditions, the ferric ions in the EPS could be reduced by the attached bacteria in the process of bioleaching of metal sulfide [127]. Rodriguezleiva and Tributsch detected that the range of the thickness of the EPS was from 10 nm to 100 nm and the EPS thickness of *At. ferrooxidans* was estimated to be 28.7 nm (±13.5) based on the analysis of AFM [128]. Ohmura et al. found the *Acidithiobacillus ferrooxidans* was more likely to attach to sulphides that contain iron [129]. Solari et al. proposed that the adhesion rate of inoculum would be elevated if the pH was reduced due to the change of the bacterial hydrophobicity in specific pH environment. Edwards and Rutenberg summarized that the small alterations of local surface in according to bacterial metabolism could strongly affect the parameters of local adhesion [130]. Flemming and Wingender presented that the formation of bacterial biofilm was accompanied by the obvious augment in production of EPS [131]. Microbial attachment and biofilm formation provide a mechanism through which the microorganism can locate itself near an energy source. It is widely accepted that the passivation of the surface of metal sulfide (e.g., chalcopyrite) is the main reason for the low leaching rate. The elemental S and jarosite are vital components for the formation. S can be formed by oxidizing the surface of sulphide and following intermediate through using Fe³⁺ and S-oxidizing

bacteria. Actually, in low redox conditions, elemental S in chalcopyrite surfaces can also be formed through reduction reactions [132]. The equations of the reduction of chalcopyrite are listed as followed,



At the middle or end of the process of bioleaching, the concentrations of Fe³⁺ and SO₄²⁻ reached at a certain height which facilitated the production of jarosite precipitation with cations like K⁺, Na⁺, NH₄⁺ or H₃O⁺ [133]. Sasaki et al. analyzed the secondary minerals with *A. ferrooxidans* by using spectroscopy, Fourier transform infrared (FT-IR) and XRD and found that the potassium jarosite was firstly found during the process of leaching, then CuS was paid attention and S was detected in the leached residue [134]. The equation of the formation of the jarosite is listed as followed,



Gonzalez et al. showed that the formation of biofilm on surfaces of sulfur or pyrite could be enhanced by adding C-14 AHL, which caused the obvious increase of EPS [15]. *A. ferrooxidans* is one of the most used bacteria for the studies on the genome and genetic information of bioleaching bacteria [135]. Some genes of *Acidithiobacillus ferrooxidans* was found resemble with those of *Escherichia coli*. Although researchers-SO₄²⁻ unremitting efforts, there are still some issues and problems about the location of the specific gene sequence and the efficient application into the industry unsolved.

4. Catalysis and galvanic interaction

It has been widely demonstrated that the combination usage of pyrite and chalcopyrite in ferric sulfate solution facilitates and increases the leaching rate compared with the use of single one [28,29,136,137]. Pyrite is considered to take the role of the catalytic properties in the process due to the function of the cathode under ambient atmosphere. During the process of GalvanoxTM, the

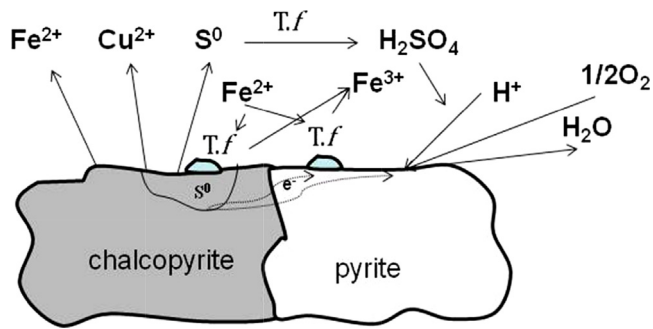
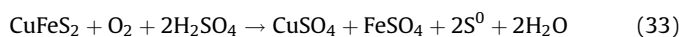
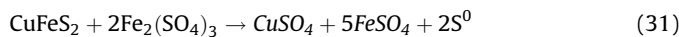
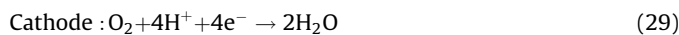
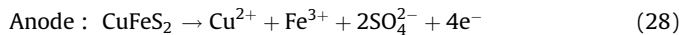


Fig. 7. The process of Galvanox™.

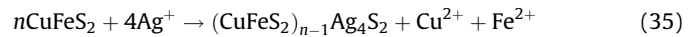
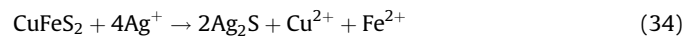
production of elemental sulfur is observed. That is caused by the oxidation of ferric ions, which complies with the polysulfate pathway. The chalcopyrite is not directly in contact with pyrite due to the existence of elemental sulfur and intermediates, and the transfer of electrons between the pyrite and chalcopyrite [138]. The process of Galvanox™ is showed as Fig. 7.

Koleini et al. presented that the ratio of the pyrite and the chalcopyrite, redox potential and temperature have significant influences on leaching rate of copper ions [139]. Dixon et al., presented that high leaching rate of copper can be reached and gotten through the Galvanox™ process which have been eventually applied into the craft of leaching or bioleaching of low-grade primary metal sulfide and deposit [28]. The equations of the Galvanox™ are listed as followed,

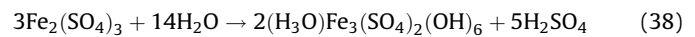
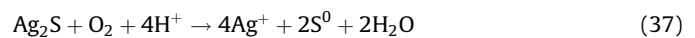
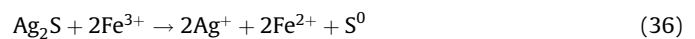


Nazari et al. proposed that that diversity and the differences of the pyrite could significantly influence the leaching rate of chalcopyrite, during the process of Galvanox™ based on the conclusion of the studies. Liang et al. found that the the leaching rate of copper was obviously improved from 64% to 95% during the process of 10 days when 2 g/L of activated carbon was added to the chalcopyrite bioleaching systems with extreme thermophile *Acidianus manzaensis* [140,141]. Activated carbon could form galvanic couples with chalcopyrite due to its conductivity and high potential. Activated carbon could accelerate and facilitate the dissolution of chalcopyrite and went through oxidation of chalcocite [65]. The role of catalyst silver has been widely studied in the chemical and biological leaching systems of chalcopyrite [142,143]. Snell and Fords displayed that the leaching rate of copper from chalcopyrite could be substantially elevated in ferric sulfate solution by adding silver ions. Miller and Portillo proposed that the production of Ag₂S film which forms on the surface of

metal sulfide (e.g., chalcopyrite) by an exchange reaction explained the catalytic effect of silver [144–146], the equation is listed as followed,



Many authors have presented that elemental sulfur film formed on the Ag₂S is porous and non-protective [142,146,147]. The catalytic effect of silver ions is accomplished by oxidation of the layer of silver sulfide under the specific redox condition. The dissolution of silver sulfide could be effectively increased when the redox is obviously elevated, which also facilitates the formation of jarosite through the ferric sulfate hydrolysis and the silver is easily wrapped in the structure of the precipitation to form argentojarosite, the related equations are listed as followed,



The activation energy of chalcopyrite was potentially reduced from 130.7 kJ mol⁻¹ to 29.3 kJ mol⁻¹ by adding silver ions [101], but not Ag⁰ [22]. The enhancement of leaching from chalcopyrite is reached through redox interactions [19,144–146] by adding the silver ions, not by the galvanic interaction of argentite due to its lower rest potential in compare with chalcopyrite. Recently, Nazari et al. presented the amazing effect and proposed the mechanism of the catalytic effects of silver-enhanced pyrite in ferric sulfate media [148,149]. Whereas, considering the relatively expensive cost and operational capital, the application of silver catalyst in leaching of chalcopyrite has the realistic difficulty in implementation.

5. Applications of biohydrometallurgy

Bioleaching is broadly used in the heap leaching of secondary copper sulfide minerals. There are some inevitable issues in respect with leaching of the primary copper sulfides due to the refractory characteristics, under ambient temperature conditions [133]. Chalcopyrite is widely studied in terms of the leaching of primary copper sulfides [20,21,133], because of the extensive resource stockpile and classic representative in the world. Mt. Lyell operation in Tasmania Australia showed the viability and considerable prospect in terms of the commercial operation by using moderately thermophilic bacteria to leach a finely ground concentrate based on the scale of pilot trial during one year. Watling et al. presented the moderately thermophilic *Sulfobacillus* bacteria were less tolerant with the concentration of soluble metal ions and also proposed the adaptability of the bacteria to the specific leaching environment, based on the bench-scale studies [20]. Bacterial growth is affected by many inhibitors in tank and heap bioleaching. The bacterial adaptation to the leaching environment could be elevated and achieved by a lengthy process of progressive pre-adapted practice to specific conditions, such as shearing stress, aeration velocity, redox, potential, temperature, pulp concentrations and pH [16,150]. The leaching bacteria in location in Chile have successfully adapted to the cold temperatures and low oxygen partial pressure resulted from the high

altitudes and the specific climatic environment. The technology of heap leaching is widely developed in Chile, with more than 85,000 t of ore processed per day. With the improvement of the industrial application, the thermophilic bacteria are considered to be indispensable for the dissolution and high copper leaching rate of refractory metal sulfide minerals in biohydrometallurgy. The extremely thermophilic archaea, due to their tolerance to extreme conditions, are eventually identified in the laboratory and applied gradually into the biohydrometallurgy, especially for the bioleaching of a highly refractory metal sulfide ores [20].

5.1. Bioleaching and biooxidation technology

The efficiency of the process of bioleaching and biooxidation is controlled by the characteristics of the metal sulfides [151]. Heaps and stirred tanks, which are two different engineering applications from traditional metallurgical industries, are mostly applied and implemented into the bioleaching and biooxidation of metal sulfides minerals in terms of biohydrometallurgy. Biohydrometallurgy is now applied on a commercial scale for the leaching of copper and the pretreatment of refractory gold ores and concentrates. BioCOP™ process is famous for the demonstration plant at Chuquicamata, in northern Chile. It produces 20,000 t of cathode copper per year by the process of the stirred-tank bioleaching and biooxidation of copper sulfides and BacTech/Mintek process. Similarly, there is also an agitated tank process used to deal with the copper sulfides, built and further developed by Bac-Tech Environment. The GEOCOAT and GEOLEACH™ processes, which both incorporate Hot Heap™ control technology, are widely used for the biooxidation or bioleaching of metal sulfide minerals through the craft of the leaching heap. The process of GEOCOAT is applicable to the biooxidation of refractory gold sulfide concentrates and to the bioleaching of copper, nickel, cobalt, zinc, and polymetallic base metal concentrates. The GEOLEACH™ technology is designed to maximize heat conservation by the control of aeration and irrigation rates, which is suitable for the whole ore systems. The general process of the heap leaching includes: the stack of metal sulfide ores on a lined pad; irrigation with the combination of a dilute sulfuric acid culture and the leaching bacteria; the control and monitor of the bioleaching conditions and environments; collection and transportation of pregnant leach solution (PLS); the processes of conventional and traditional metal extraction and electrowinning. The mineral ores that are used for stack or heap usually are pre-treated by crushing or grinding into the specific sizes. Considering the aeration of the leaching heap and the limitation of natural convection, the gangues are used for the acid agglomeration (the GEOCOAT process) and sometimes the lines are deployed on the pad under the stack to supply the oxygen (O₂) and carbon dioxide (CO₂). The chemolithotrophic and acidophilic bacteria fix carbon dioxide and obtain energy from the oxidation of ferrous iron or reduced sulfur compounds by the oxygen as the electron acceptor [152–154]. It is generally demonstrated that the discontinuous irrigation facilitates the dissolution of metal sulfide mineral due to the functions of the capillary forces of the ores. The frequency of irrigation is determined by the rate of evaporation and the concentration of the metal at the bottom of the heap [155,156]. Sometimes the heap is formed with multi-deck stacking according to the scale of the mineral distribution. Stirred tank leaching involves finer particle sizes agitated in the cultural solution and usually is applied to the mineral concentrates for high-value metals due to its higher capital and operating costs than that of the heap leaching. The leaching heap and stirred-tank bioreactors are heated by the exothermic process of the biooxidation of metal sulfide particles. The height of the leaching heap or the amount of ores in the tank is a vital factor to the temperature, considering the cost and engineering difficulty

in the control of the temperature during the leaching process. Petersen and Dixon presented that the temperature inside the heap of chalcopyrite was influenced by many factors, such as oxidation and biooxidation rate of the sulfide, aeration and the rates of irrigation, humidity of the air, solar radiation and evaporation [32]. There are more factors which cannot be effectively controlled when comparing with tank reactors, such as the organization of the systematic aeration, the control of pH and nutrient levels, adjustment of the channels of the gaseous (O₂, CO₂) and liquid (nutrient solution) transportation [157]. In terms of the process of the stirred-tank bioleaching and biooxidation, the mineral ores are pre-treated with conventional mining methods, flotation or gravity separation. The pulp metal concentration, physical damage resulted from the shear force of blade and fictions of particles, the stresses and the velocity of agitation and aeration to the leaching bacteria should be additionally considered in design and implementation of the stirred-tank bioreactors. Totally, the microorganisms detected in heap leaching and stirred-tank processes are similar in terms of the types, while the proportion of the bacteria varied based on the specific and different craft conditions.

5.2. Chloride application in the bioleaching process

The effects of chloride on the process of the leaching and bioleaching of metal sulfide have been extensively studied for decades while the detailed mechanisms on the beneficial role of low chloride in leaching system remain blurry and incomplete, especially about the process of interactions on leaching bacteria based on the molecular biology scale [158,159]. It is widely demonstrated that the leaching rate of metal sulfides can be higher in chloride leaching solution than that in sulphate media solution. Dutrizac and MacDonald investigated the dissolution of chalcopyrite under dump leaching with addition of chloride (NaCl, 6 g/L) and found that the process of the leaching in low-grade metal sulfide ores was facilitated [160]. Kinnunen and Puhakka proposed the change amplitude of the leaching temperature would distinctly affect the leaching kinetics in the chloride media solution [161]. He found the production of copper ions was enhanced from 67 °C to 90 °C under the condition of 0.25 g/L of Cl⁻ concentrate but was descended at 50 °C. The leaching rates of chalcopyrite in ferric-chloride media solution found to be faster than that in media solution of ferric-sulfate. The rational analysis was the exist of the chloride in the leaching solution caused the formation of a crystalline and more porous sulfur layer, not the amorphous or cryptocrystalline film as the second phase under the absence of chloride [140]. The second phases produced during the leaching process, such as elemental sulfur, covellite, chalcocite and jarosite, contribute to the passivation layer on the surface of chalcopyrite. Carneiro and Leão found the porosity of secondary phase layer was expanded when 0.5–2.0 M Na-chloride was added into the chalcopyrite leaching solution. Liang et al. presented that the accumulation quantity of elemental sulfur was substantially reduced with 11 mM sodium Na-chloride in the chalcopyrite thermophilic bioleaching solution (65 °C) [140]. Cai et al. detected the production of the covellite in chloride leaching solution during the process of chalcopyrite dissolution [162]. Cu⁺ is monovalent in the band structure of chalcopyrite and its dissolution could easily be elevated by the formation of soluble Cu⁺-Cl⁻ complexes. The impact of chloride on the growth of bioleaching strains has been broadly reported, such as *A. ferrooxidans*, *L. ferriphilum*, *S. metallicus*, *S. rivotincti* [163] and a mixed mesophilic culture [164]. It was obviously detected that a certain amount of chloride in the leaching solution would inhibit the growth of the iron- and sulfur-oxidizing microorganisms [165] and chloride toxicity to microorganisms displayed explicit differences and multiformalities. Harahuc et al. presented that the growth of iron-grown

Acidithiobacillus ferrooxidans was locally inhibited at the condition of 10 mM KCl and sulfur-grown bacteria could tolerate up to 200 mM [165]. Shiers et al. showed that concentrations of 7 g/L NaCl reduced cell replication by 50% and that no significant culture adaptation or habituation occurred with prolonged exposure to that concentration [164]. Deveci et al. reported that salinity in the range of 1–4% (NaCl w/v) was substantially detrimental to mesophilic bioleaching microorganisms [166]. Gahan et al. found that chloride at 4 g/L (110 mM) was lethal to a pyrite-oxidizing microbial consortium [167]. During the process of bioleaching and biooxidation, the amount and species of chloride that will be applied into the bioreactors must be calculated and tested by bench and pilot experiments to balance the benefits of chloride and the inhibition of the microbial population, which is an inevitable issue in industrial application of biohydrometallurgy.

Acknowledgements

This study is financially supported by the National Natural Science Foundation of China (No. 51274262) and National Engineering Research Center of Phosphate Resources Development and Utilization Foundation of China (No. 2012 National Phosphate k002).

References

- [1] A. Sandstrom, S. Petersson, Bioleaching of a complex sulphide ore with moderate thermophilic and extreme thermophilic microorganisms, *Hydrometallurgy* 46 (1–2) (1997) 181–190.
- [2] B. Fu, H.B. Zhou, R.B. Zhang, G.Z. Qiu, Bioleaching of chalcopyrite by pure and mixed cultures of *Acidithiobacillus* spp. and *Leptospirillum ferriphilum*, *Int. Biodeter. Biodegr.* 62 (2) (2008) 109–115.
- [3] J. Li, N. Kawashima, K. Kaplun, V.J. Absolon, A.R. Gerson, Chalcopyrite leaching: the rate controlling factors, *Geochim. Cosmochim. Acta* 74 (10) (2010) 2881–2893.
- [4] P.K. Abraitis, R.A.D. Pattrick, G.H. Kelsall, D.J. Vaughan, Acid leaching and dissolution of major sulphide ore minerals: processes and galvanic effects in complex systems, *Min. Mag.* 68 (2) (2004) 343–351.
- [5] K. Bosecker, Bioleaching: metal solubilization by microorganisms, *Fems Microbiol. Rev.* 20 (3–4) (1997) 591–604.
- [6] G.J. Olson, J.A. Brierley, C.L. Brierley, Bioleaching review part B: progress in bioleaching: applications of microbial processes by the minerals industries, *Appl. Microbiol. Biotechnol.* 63 (3) (2003) 249–257.
- [7] A. Akcil, Potential bioleaching developments towards commercial reality: Turkish metal mining's future, *Miner. Eng.* 17 (3) (2004) 477–480.
- [8] M.E. Clark, J.D. Batty, C.B. van Buuren, D.W. Dew, M.A. Eamon, Biotechnology in minerals processing: technological breakthroughs creating value, *Hydrometallurgy* 83 (1–4) (2006) 3–9.
- [9] D.B. Johnson, Geomicrobiology of extremely acidic subsurface environments, *Fems Microbiol. Ecol.* 81 (1) (2012) 2–12.
- [10] D.B. Johnson, K.B. Hallberg, Acid mine drainage remediation options: a review, *Sci. Total Environ.* 338 (1–2) (2005) 3–14.
- [11] W. Sand, T. Gehrke, P.G. Jozsa, A. Schippers, (Bio) chemistry of bacterial leaching – direct vs. indirect bioleaching, *Hydrometallurgy* 59 (2–3) (2001) 159–175.
- [12] D.E. Rawlings, Heavy metal mining using microbes, *Annu. Rev. Microbiol.* 56 (2002) 65–91.
- [13] T. Rohwerder, T. Gehrke, K. Kinzler, W. Sand, Bioleaching review part A: progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation, *Appl. Microbiol. Biotechnol.* 63 (3) (2003) 239–248.
- [14] L.M. Ruiz, S. Valenzuela, M. Castro, A. Gonzalez, M. Frezza, L. Souler, T. Rohwerder, Y. Queneau, A. Doutheau, W. Sand, C.A. Jerez, N. Guiliani, AHL communication is a widespread phenomenon in biomining bacteria and seems to be involved in mineral-adhesion efficiency, *Hydrometallurgy* 94 (1–4) (2008) 133–137.
- [15] A. Gonzalez, S. Bellenberg, S. Mamani, L. Ruiz, A. Echeverria, L. Souler, A. Doutheau, C. Demergasso, W. Sand, Y. Queneau, M. Vera, N. Guiliani, AHL signaling molecules with a large acyl chain enhance biofilm formation on sulfur and metal sulfides by the bioleaching bacterium *Acidithiobacillus ferrooxidans*, *Appl. Microbiol. Biotechnol.* 97 (8) (2013) 3729–3737.
- [16] L.X. Xia, X.X. Liu, J. Zeng, C. Yin, J. Gao, J.S. Liu, G.Z. Qu, Mechanism of enhanced bioleaching efficiency of *Acidithiobacillus ferrooxidans* after adaptation with chalcopyrite, *Hydrometallurgy* 92 (3–4) (2008) 95–101.
- [17] Y. Rodriguez, A. Ballester, M.L. Blazquez, F. Gonzalez, J.A. Munoz, Study of bacterial attachment during the bioleaching of pyrite, chalcopyrite, and sphalerite, *Geomicrobiol. J.* 20 (2) (2003) 131–141.
- [18] A. Sanhueza, I.J. Ferrer, T. Vargas, A. Amils, C. Sanchez, Attachment of *Thiobacillus ferrooxidans* on synthetic pyrite of varying structural and electronic properties, *Hydrometallurgy* 51 (1) (1999) 115–129.
- [19] N. Hiroyoshi, M. Arai, H. Miki, M. Tsunekawa, T. Hirajima, A new reaction model for the catalytic effect of silver ions on chalcopyrite leaching in sulfuric acid solutions, *Hydrometallurgy* 63 (3) (2002) 257–267.
- [20] H.R. Watling, The bioleaching of sulphide minerals with emphasis on copper sulphides – a review, *Hydrometallurgy* 84 (1–2) (2006) 81–108.
- [21] H.R. Watling, F.A. Perrot, D.W. Shiers, Comparison of selected characteristics of *Sulfobacillus* species and review of their occurrence in acidic and bioleaching environments, *Hydrometallurgy* 93 (1–2) (2008) 57–65.
- [22] C. Klauber, A. Parker, W. van Bronswijk, H. Watling, Sulphur speciation of leached chalcopyrite surfaces as determined by X-ray photoelectron spectroscopy, *Int. J. Miner. Process.* 62 (1–4) (2001) 65–94.
- [23] J.E. Dutrizac, Elemental sulfur formation during the ferric sulfate leaching of chalcopyrite, *Can. Metall. Q.* 28 (4) (1989) 337–344.
- [24] Q. Yin, G.H. Kelsall, D.J. Vaughan, K.E.R. England, Atmospheric and electrochemical oxidation of the surface of chalcopyrite (Cufes₂), *Geochim. Cosmochim. Acta* 59 (6) (1995) 1091–1100.
- [25] V.L. Tauson, R.G. Kravtsova, V.I. Grebenshchikova, E.E. Lustenberg, S.V. Lipko, Surface typochemistry of hydrothermal pyrite: electron spectroscopic and scanning probe microscopic data. II. Natural pyrite, *Geochem. Int.* 47 (3) (2009) 231–243.
- [26] D. Rickard, G.W. Luther, Chemistry of iron sulfides, *Chem. Rev.* 107 (2) (2007) 514–562.
- [27] A.K. Kleppe, A.P. Jephcoat, High-pressure Raman spectroscopic studies of FeS₂ pyrite, *Miner. Mag.* 68 (3) (2004) 433–441.
- [28] D.G. Dixon, D.D. Mayne, K.G. Baxter, Galvanox (TM) – a novel galvanically-assisted atmospheric leaching technology for copper concentrates, *Can. Metall. Q.* 47 (3) (2008) 327–336.
- [29] J.A. Munoz, D.B. Dreisinger, W.C. Cooper, S.K. Young, Interaction of silver ions with sulphide minerals with special emphasis on the chalcopyrite/pyrite galvanic couple, *Can. Metall. Q.* 47 (3) (2008) 259–268.
- [30] A. Azizi, C.F. Petre, C. Olsen, F. Larachi, Untangling galvanic and passivation phenomena induced by sulfide minerals on precious metal leaching using a new packed-bed electrochemical cyanidation reactor, *Hydrometallurgy* 107 (3–4) (2011) 101–111.
- [31] G.A. Padilla, L.A. Cisternas, J.Y. Cueto, On the optimization of heap leaching, *Miner. Eng.* 21 (9) (2008) 673–678.
- [32] J. Petersen, D.G. Dixon, Thermophilic heap leaching of a chalcopyrite concentrate, *Miner. Eng.* 15 (11) (2002) 777–785.
- [33] J. Petersen, D.G. Dixon, Modelling zinc heap bioleaching, *Hydrometallurgy* 85 (2–4) (2007) 127–143.
- [34] S. Grazulis, D. Chateigner, R.T. Downs, A.F.T. Yokochi, M. Quiros, L. Lutterotti, E. Manakova, J. Butkus, P. Moeck, A. Le Bail, Crystallography open database – an open-access collection of crystal structures, *J. Appl. Crystallogr.* 42 (2009) 726–729.
- [35] K.G. Nikiforov, Magnetically ordered multinary semiconductors, *Prog. Cryst. Growth Charact.* 39 (1–4) (1999) 1–104.
- [36] C.L. Burdick, J.H. Ellis, The crystal structure of chalcopyrite determined by X-rays, *J. Am. Chem. Soc.* 39 (1917) 2518–2525.
- [37] S.R. Hall, J.F. Rowland, Crystal-structure of synthetic mooioheokite Cu₉Fe₉S₁₆, *Acta Crystallogr. B* 29 (November 15) (1973) 2365–2372.
- [38] S.R. Hall, J.M. Stewart, Crystal-structure refinement of chalcopyrite, Cufes₂, *Acta Crystallogr. B* 29 (15) (1973) 579–585.
- [39] A. Hung, J. Muscat, I. Yarovsky, S.P. Russo, Density-functional theory studies of pyrite FeS₂ (100) and (110) surfaces, *Surf. Sci.* 513 (3) (2002) 511–524.
- [40] A. Hung, J. Muscat, I. Yarovsky, S.P. Russo, Density-functional theory studies of pyrite FeS₂ (111) and (210) surfaces, *Surf. Sci.* 520 (1–2) (2002) 111–119.
- [41] Y. Konishi, H. Kubo, S. Asai, Bioleaching of zinc-sulfide concentrate by *Thiobacillus ferrooxidans*, *Biotechnol. Bioeng.* 39 (1) (1992) 66–74.
- [42] J. Cai, M.R. Philpott, Electronic structure of bulk and (001) surface layers of pyrite FeS₂, *Comp. Mater. Sci.* 30 (3–4) (2004) 358–363.
- [43] M. Fujisawa, S. Suga, T. Mizokawa, A. Fujimori, K. Sato, Electronic-structures of Cufes₂ and Cu₁₁(0.9)Fe(0.1)S₂ studied by electron and optical spectroscopies, *Phys. Rev. B* 49 (11) (1994) 7155–7164.
- [44] Y. Mikhlin, Y. Tomashevich, V. Tauson, D. Vyalikh, S. Molodtsov, R. Szargan, A comparative X-ray absorption near-edge structure study of bornite, Cu₅FeS₄, and chalcopyrite, CuFeS₂, *J. Electron Spectrosc.* 142 (1) (2005) 83–88.
- [45] T. Oguchi, K. Sato, T. Teranishi, Optical reflectivity spectrum of a Cufes₂ single-crystal, *J. Phys. Soc. Jpn.* 48 (1) (1980) 123–128.
- [46] J.C. Woolley, A.M. Lamarche, G. Lamarche, M. Quintero, I.P. Swainson, T.M. Holden, Low temperature magnetic behaviour of CuFeS₂ from neutron diffraction data, *J. Magn. Magn. Mater.* 162 (2–3) (1996) 347–354.
- [47] A.M. Lamarche, J.C. Woolley, G. Lamarche, I.P. Swainson, T.M. Holden, Structure and magnetic properties of the ternary compound copper iron telluride, *J. Magn. Magn. Mater.* 1–2 (1998) 121–128.
- [48] J. Petiau, P. Saintavit, G.K. Calas, X-Ray absorption-spectra and electronic-structure of chalcopyrite Cufes₂, *Mat. Sci. Eng. B-Solid* 1 (3–4) (1988) 237–249.
- [49] D. Raj, K. Chandra, S.P. Puri, Mossbauer studies of chalcopyrite, *J. Phys. Soc. Jpn.* 24 (1) (1968) 39.
- [50] C.I. Pearce, R.A.D. Pattrick, D.J. Vaughan, C.M.B. Henderson, G. van der Laan, Copper oxidation state in chalcopyrite: mixed Cu d(9) and d(10) characteristics, *Geochim. Cosmochim. Acta* 70 (18) (2006) 4635–4642.
- [51] C. de Oliveira, G.F. de Lima, H.A. de Abreu, H.A. Duarte, Reconstruction of the chalcopyrite surfaces – a DFT study, *J. Phys. Chem. C* 116 (10) (2012) 6357–6366.
- [52] C. de Oliveira, H.A. Duarte, Disulphide and metal sulphide formation on the reconstructed (001) surface of chalcopyrite: a DFT study, *Appl. Surf. Sci.* 257 (4) (2010) 1319–1324.

- [53] J.C.W. Folmer, F. Jelinek, G.H.M. Calis, The electronic-structure of pyrites, particularly CuS and $\text{Fe}_1\text{-Xcuxse}_2$ - an Xps and mossbauer study, *J. Solid State Chem.* 1 (1988) 137–144.
- [54] R. Edelbro, A. Sandstrom, J. Paul, Full potential calculations on the electron bandstructures of sphalerite, pyrite and chalcopyrite, *Appl. Surf. Sci.* 206 (1–4) (2003) 300–313.
- [55] K.M. Rosso, U. Becker, M.F. Hochella, Atomically resolved electronic structure of pyrite {100} surfaces: an experimental and theoretical investigation with implications for reactivity, *Am. Mineral.* 84 (10) (1999) 1535–1548.
- [56] K.M. Rosso, U. Becker, M.F. Hochella, The interaction of pyrite {100} surfaces with O_2 and H_2O : fundamental oxidation mechanisms, *Am. Mineral.* 84 (10) (1999) 1549–1561.
- [57] G.Z. Qiu, Q. Xiao, Y.H. Hu, W.Q. Qin, D.Z. Wang, Theoretical study of the surface energy and electronic structure of pyrite FeS_2 (100) using a total-energy pseudopotential method, *CASTEP, J. Colloid Interf. Sci.* 270 (1) (2004) 127–132.
- [58] S.L. Harmer, A.R. Pratt, W.H. Nesbitt, M.E. Fleet, Sulfur species at chalcopyrite (CuFeS_2) fracture surfaces, *Am. Mineral.* 89 (7) (2004) 1026–1032.
- [59] H.W. Nesbitt, A.G. Berlich, S.L. Harmer, I. Uhlig, G.M. Bancroft, R. Szargan, Identification of pyrite valence band contributions using synchrotron-excited X-ray photoelectron spectroscopy, *Am. Mineral.* 89 (2–3) (2004) 382–389.
- [60] R.G. Acres, S.L. Harmer, D.A. Beattie, Synchrotron XPS, NEXAFS, and ToF-SIMS studies of solution exposed chalcopyrite and heterogeneous chalcopyrite with pyrite, *Miner. Eng.* 23 (11–13) (2010) 928–936.
- [61] R.G. Acres, S.L. Harmer, H.W. Shui, C.H. Chen, D.A. Beattie, Synchrotron scanning photoemission microscopy of homogeneous and heterogeneous metal sulfide minerals, *J. Synchrotron Radiat.* 18 (2011) 649–657.
- [62] G.U. Von Oertzen, S.L. Harmer, W.M. Skinner, XPS and ab initio calculation of surface states of sulfide minerals: pyrite, chalcopyrite and molybdenite, *Mol. Simul.* 32 (15) (2006) 1207–1212.
- [63] G.U. von Oertzen, W.M. Skinner, H.W. Nesbitt, Ab initio and XPS studies of pyrite (100) surface states, *Radiat. Phys. Chem.* 75 (11) (2006) 1855–1860.
- [64] C. Klauber, Fracture-induced reconstruction of a chalcopyrite (CuFeS_2) surface, *Surf. Interface Anal.* 35 (5) (2003) 415–428.
- [65] M.L. Liu, F.Q. Huang, L.D. Chen, Y.M. Wang, Y.H. Wang, G.F. Li, Q. Zhang, *p*-type transparent conductor: Zn-doped CuAlS_2 , *Appl. Phys. Lett.* 90 (7) (2007).
- [66] A.N. Mariano, R.M. Beger, Cleavage in pyrite and cobaltite, *Am. Miner.* 56 (11–1) (1971) 1867–1881.
- [67] C. Pettenkofer, W. Jaegermann, M. Bronold, Site specific surface interaction of electron-donors and acceptors on FeS_2 (100) cleavage planes, *Ber. Bunsen. Phys. Chem.* 95 (5) (1991) 560–565.
- [68] M. Bronold, Y. Tamm, W. Jaegermann, Surface-states on cubic D-band semiconductor pyrite (FeS_2), *Surf. Sci.* 314 (3) (1994) L931–L936.
- [69] H.W. Nesbitt, G.M. Bancroft, A.R. Pratt, M.J. Scaini, Sulfur and iron surface states on fractured pyrite surfaces, *Am. Mineral.* 83 (9–10) (1998) 1067–1076.
- [70] J.A. Leiro, S.S. Mattila, K. Laajalehto, XPS study of the sulphur 2p spectra of pyrite, *Surf. Sci.* 547 (1–2) (2003) 157–161.
- [71] J. Alonso-Azcarate, M. Rodas, L. Fernandez-Diaz, S.H. Bottrell, J.R. Mas, S. Lopez-Andres, Causes of variation in crystal morphology in metamorphogenic pyrite deposits of the Cameros Basin (N Spain), *Geol. J.* 36 (2) (2001) 159–170.
- [72] P.K. Abratis, R.A.D. Patrick, D.J. Vaughan, Variations in the compositional, textural and electrical properties of natural pyrite: a review, *Int. J. Miner. Process.* 74 (1–4) (2004) 41–59.
- [73] M. Gericke, Y. Govender, Bioremediation strategies for the treatment of nickel-copper sulphide concentrates, *Miner. Eng.* 24 (11) (2011) 1106–1112.
- [74] D.B. Johnson, Biomining—biotechnologies for extracting and recovering metals from ores and waste materials, *Curr. Opin. Biotechnol.* 30C (2014) 24–31.
- [75] B. Dold, Mineralogical and Geochemical Controls in Biomining and Bioremediation, in: N. Parmar, A. Singh (Eds.), *Geomicrobiology and Biogeochemistry*, Springer, Berlin, Heidelberg, 2014, pp. 119–135.
- [76] K.B. Hallberg, D.B. Johnson, Novel acidophiles isolated from moderately acidic mine drainage waters, *Hydrometallurgy* 71 (1–2) (2003) 139–148.
- [77] D.B. Johnson, K.B. Hallberg, The microbiology of acidic mine waters, *Res. Microbiol.* 154 (7) (2003) 466–473.
- [78] S. Hedrich, M. Schlomann, D.B. Johnson, The iron-oxidizing proteobacteria, *Microbiol-Sgm* 157 (2011) 1551–1564.
- [79] D.B. Johnson, Biodiversity and ecology of acidophilic microorganisms, *Fems Microbiol. Ecol.* 27 (4) (1998) 307–317.
- [80] M. Dopson, D.B. Johnson, Biodiversity, metabolism and applications of acidophilic sulfur-metabolizing microorganisms, *Environ. Microbiol.* 14 (10) (2012) 2620–2631.
- [81] D.A. Clark, P.R. Norris, Acidimicrobium ferrooxidans gen nov, sp nov: mixed-culture ferrous iron oxidation with *Sulfobacillus* species, *Microbiol-Uk* 142 (1996) 785–790.
- [82] H. Tributsch, Direct versus indirect bioleaching, *Hydrometallurgy* 59 (2–3) (2001) 177–185.
- [83] F.K. Crundwell, How do bacteria interact with minerals? *Hydrometallurgy* 71 (1–2) (2003) 75–81.
- [84] A. Schippers, W. Sand, Bacterial leaching of metal sulfides proceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur, *Appl. Environ. Microbiol.* 65 (1) (1999) 319–321.
- [85] A.N. Buckley, R. Woods, The surface oxidation of pyrite, *Appl. Surf. Sci.* 27 (4) (1987) 437–452.
- [86] C.O. Moses, D.K. Nordstrom, J.S. Herman, A.L. Mills, Aqueous pyrite oxidation by dissolved-oxygen and by ferric iron, *Geochim. Cosmochim. Acta* 51 (6) (1987) 1561–1571.
- [87] G. Druschel, M. Borda, Comment on Pyrite dissolution in acidic media by M. Descostes, P. Vitorge, and C. Beaucaire, *Geochim. Cosmochim. Acta* 70 (20) (2006) 5246–5250.
- [88] E.C. Todd, D.M. Sherman, J.A. Purton, Surface oxidation of chalcopyrite (CuFeS_2) under ambient atmospheric and aqueous (pH 2–10) conditions: Cu, Fe L- and OK-edge X-ray spectroscopy, *Geochim. Cosmochim. Acta* 67 (12) (2003) 2137–2146.
- [89] P.C. Singer, W. Stumm, Direct oxidation by adsorbed oxygen during acidic mine drainage, *Science* 169 (3940) (1970) 98.
- [90] P.R. Holmes, F.K. Crundwell, The kinetics of the oxidation of pyrite by ferric ions and dissolved oxygen: an electrochemical study, *Geochim. Cosmochim. Acta* 64 (2) (2000) 263–274.
- [91] Y. Xu, M.A.A. Schoonen, The stability of thiosulfate in the presence of pyrite in low-temperature aqueous-solutions, *Geochim. Cosmochim. Acta* 59 (22) (1995) 4605–4622.
- [92] A. Schippers, P.G. Jozsa, W. Sand, Sulfur chemistry in bacterial leaching of pyrite, *Appl. Environ. Microbiol.* 62 (9) (1996) 3424–3431.
- [93] M.A. Williamson, J.D. Rimstidt, The kinetics and electrochemical rate-determining step of aqueous pyrite oxidation, *Geochim. Cosmochim. Acta* 58 (24) (1994) 5443–5454.
- [94] M. Vera, A. Schippers, W. Sand, Progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation—part A, *Appl. Microbiol. Biotechnol.* 97 (17) (2013) 7529–7541.
- [95] N. Balci, B. Mayer, W.C. Shanks, K.W. Mandernack, Oxygen and sulfur isotope systematics of sulfate produced during abiotic and bacterial oxidation of sphalerite and elemental sulfur, *Geochim. Cosmochim. Acta* 77 (2012) 335–351.
- [96] N. Balci, W.C. Shanks, B. Mayer, K.W. Mandernack, Oxygen and sulfur isotope systematics of sulfate produced by bacterial and abiotic oxidation of pyrite, *Geochim. Cosmochim. Acta* 71 (15) (2007) 3796–3811.
- [97] T. Hirato, H. Majima, Y. Awakura, The leaching of chalcopyrite with ferric sulfate, *Metall. Trans. B* 18 (3) (1987) 489–496.
- [98] H. Majima, Y. Awakura, T. Hirato, T. Tanaka, The leaching of chalcopyrite in ferric-chloride and ferric sulfate-solutions, *Can. Metall. Q.* 24 (4) (1985) 283–291.
- [99] M.M. McGuire, K.J. Edwards, J.F. Banfield, R.J. Hamers, Kinetics, surface chemistry, and structural evolution of microbially mediated sulfide mineral dissolution, *Geochim. Cosmochim. Acta* 65 (8) (2001) 1243–1258.
- [100] M.F.C. Cameiro, V.A. Leao, The role of sodium chloride on surface properties of chalcopyrite leached with ferric sulphate, *Hydrometallurgy* 87 (3–4) (2007) 73–82.
- [101] E.M. Cordoba, J.A. Munoz, M.L. Blazquez, F. Gonzalez, A. Ballester, Leaching of chalcopyrite with ferric ion. Part I: general aspects, *Hydrometallurgy* 93 (3–4) (2008) 81–87.
- [102] D. Nava, I. Gonzalez, Electrochemical characterization of chemical species formed during the electrochemical treatment of chalcopyrite in sulfuric acid, *Electrochim. Acta* 51 (25) (2006) 5295–5303.
- [103] G.K. Parker, R. Woods, G.A. Hope, Raman investigation of chalcopyrite oxidation, *Colloid Surf. A* 318 (1–3) (2008) 160–168.
- [104] A. Ghahremanizhad, E. Asselin, D.G. Dixon, Electrochemical evaluation of the surface of chalcopyrite during dissolution in sulfuric acid solution, *Electrochim. Acta* 55 (18) (2010) 5041–5056.
- [105] R.P. Hackl, D.B. Dreisinger, E. Peters, J.A. King, Passivation of chalcopyrite during oxidative leaching in sulfate media, *Hydrometallurgy* 39 (1–3) (1995) 25–48.
- [106] G.W. Warren, M.E. Wadsworth, S.M. Elraghy, Passive and transpassive anodic behavior of chalcopyrite in acid-solutions, *Metall. Trans. B* 13 (4) (1982) 571–579.
- [107] R.S. Thurston, K.W. Mandernack, W.C. Shanks, Laboratory chalcopyrite oxidation by *Acidithiobacillus ferrooxidans*: oxygen and sulfur isotope fractionation, *Chem. Geol.* 269 (3–4) (2010) 252–261.
- [108] K. Rohwerder, W. Sand, The sulfane sulfur of polysulfides is the actual substrate of the sulfur-oxidizing enzymes from *Acidithiobacillus* and *Acidiphilium* spp, *Microbiol-Sgm* 149 (2003) 1699–1709.
- [109] W.M. Zeng, G.Z. Qiu, H.B. Zhou, M.A. Chen, Electrochemical behaviour of massive chalcopyrite electrodes bioleached by moderately thermophilic microorganisms at 48 degrees C, *Hydrometallurgy* 105 (3–4) (2011) 259–263.
- [110] H.L. Yang, S.S. Feng, Y. Xin, W. Wang, Community dynamics of attached and free cells and the effects of attached cells on chalcopyrite bioleaching by *Acidithiobacillus* sp, *Bioresour. Technol.* 154 (2014) 185–191.
- [111] K. Harneit, A. Goksel, D. Kock, J.H. Klock, T. Gehrke, W. Sand, Adhesion to metal sulfide surfaces by cells of *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans*, *Hydrometallurgy* 83 (1–4) (2006) 245–254.
- [112] S. Bellenberg, C.F. Leon-Morales, W. Sand, M. Vera, Visualization of capsular polysaccharide induction in *Acidithiobacillus ferrooxidans*, *Hydrometallurgy* 129 (2012) 82–89.
- [113] M. Vera, B. Krok, S. Bellenberg, W. Sand, A. Poetsch, Shotgun proteomics study of early biofilm formation process of *Acidithiobacillus ferrooxidans* ATCC 23,270 on pyrite, *Proteomics* 13 (7) (2013) 1133–1144.
- [114] R.C. Blake, M.N. Griff, In situ spectroscopy on intact *Leptospirillum ferrooxidans* reveals that reduced cytochrome 579 is an obligatory intermediate in the aerobic iron respiratory chain, *Front Microbiol.* 3 (2012).

- [115] R.C. Blake, E.A. Shute, G.T. Howard, Solubilization of minerals by bacteria – electrophoretic mobility of *Thiobacillus ferrooxidans* in the presence of iron, pyrite, and sulfur, *Appl. Environ. Microbiol.* 60 (9) (1994) 3349–3357.
- [116] E. Gomez, M.L. Blazquez, A. Ballester, F. Gonzalez, Study by SEM and EDS of chalcopyrite bioleaching using a new thermophilic bacteria, *Miner. Eng.* 9 (9) (1996) 985–999.
- [117] G. Meyer, T. Schneider-Merck, S. Bohme, W. Sand, A simple method for investigations on the chemotaxis of *Acidithiobacillus ferrooxidans* and *Desulfovibrio vulgaris*, *Acta Biotechnol.* 22 (3–4) (2002) 391–399.
- [118] J.D. Rimstidt, D.J. Vaughan, Pyrite oxidation: a state-of-the-art assessment of the reaction mechanism, *Geochim. Cosmochim. Acta* 67 (5) (2003) 873–880.
- [119] V. Gautier, B. Escobar, T. Vargas, Cooperative action of attached and planktonic cells during bioleaching of chalcopyrite with *Sulfolobus metallicus* at 70 degrees C, *Hydrometallurgy* 94 (1–4) (2008) 121–126.
- [120] S.W. Singer, B.K. Erickson, N.C. VerBerkmoes, M. Hwang, M.B. Shah, R.L. Hettich, J.F. Banfield, M.P. Thelen, Posttranslational modification and sequence variation of redox-active proteins correlate with biofilm life cycle in natural microbial communities, *ISME J.* 4 (11) (2010) 1398–1409.
- [121] C. Castelle, M. Guiral, G. Malarte, F. Ledgham, G. Leroy, M. Brugna, M.T. Giudici-Ortoni, A new iron-oxidizing/O₂-reducing supercomplex spanning both inner and outer membranes, isolated from the extreme acidophile *Acidithiobacillus ferrooxidans*, *J. Biol. Chem.* 25 (2008) 803–25811.
- [122] A. Amouric, C. Brochier-Armanet, D.B. Johnson, V. Bonnefoy, K.B. Hallberg, Phylogenetic and genetic variation among Fe(II)-oxidizing acidithiobacilli supports the view that these comprise multiple species with different ferrous iron oxidation pathways, *Microbiol-Sgm* 157 (2011) 111–122.
- [123] T. Gehrke, R. Hallmann, K. Kinzler, W. Sand, The EPS of *Acidithiobacillus ferrooxidans* – a model for structure–function relationships of attached bacteria and their physiology, *Water Sci. Technol.* 43 (6) (2001) 159–167.
- [124] T. Gehrke, J. Telegdi, D. Thierry, W. Sand, Importance of extracellular polymeric substances from *Thiobacillus ferrooxidans* for bioleaching, *Appl. Environ. Microb.* 64 (7) (1998) 2743–2747.
- [125] P.K. Sharma, A. Das, K.H. Rao, K.S.E. Forssberg, Surface characterization of *Acidithiobacillus ferrooxidans* cells grown under different conditions, *Hydrometallurgy* 71 (1–2) (2003) 285–292.
- [126] R. Arredondo, A. Garcia, C.A. Jerez, Partial removal of lipopolysaccharide from *Thiobacillus ferrooxidans* affects its adhesion to solids, *Appl. Environ. Microbiol.* 60 (8) (1994) 2846–2851.
- [127] J.T. Pronk, J.C. Debruyne, P. Bos, J.G. Kuenen, Anaerobic growth of *Thiobacillus ferrooxidans*, *Appl. Environ. Microbiol.* 58 (7) (1992) 2227–2230.
- [128] M. Rodriguezleiva, H. Tributsch, Morphology of bacterial leaching patterns by *Thiobacillus ferrooxidans* on synthetic pyrite, *Arch. Microbiol.* 149 (5) (1988) 401–405.
- [129] N. Ohmura, K. Kitamura, H. Saiki, Selective adhesion of *Thiobacillus ferrooxidans* to pyrite, *Appl. Environ. Microbiol.* 59 (12) (1993) 4044–4050.
- [130] K.J. Edwards, A.D. Rutenberg, Microbial response to surface microtopography: the role of metabolism in localized mineral dissolution, *Chem. Geol.* 180 (1–4) (2001) 19–32.
- [131] H.C. Flemming, J. Wingender, The biofilm matrix, *Nat. Rev. Microbiol.* 8 (9) (2010) 623–633.
- [132] J. Vilcaez, C. Inoue, Mathematical modeling of thermophilic bioleaching of chalcopyrite, *Miner. Eng.* 22 (11) (2009) 951–960.
- [133] N. Pradhan, K.C. Nathsarma, K.S. Rao, L.B. Sukla, B.K. Mishra, Heap bioleaching of chalcopyrite: a review, *Miner. Eng.* 21 (5) (2008) 355–365.
- [134] K. Sasaki, Y. Nakamura, T. Hirajima, O.H. Tuovinen, Raman characterization of secondary minerals formed during chalcopyrite leaching with *Acidithiobacillus ferrooxidans*, *Hydrometallurgy* 95 (1–2) (2009) 153–158.
- [135] M. Barreto, R. Quatrini, S. Bueno, C. Arriagada, J. Valdes, S. Silver, E. Jedlicki, D.S. Holmes, Aspects of the predicted physiology of *Acidithiobacillus ferrooxidans* deduced from an analysis of its partial genome sequence, *Hydrometallurgy* 71 (1–2) (2003) 97–105.
- [136] A.P. Mehta, L.E. Murr, Fundamental-studies of the contribution of galvanic interaction to acid-bacterial leaching of mixed metal sulfides, *Hydrometallurgy* 9 (3) (1983) 235–256.
- [137] P. Nowak, E. Krauss, A. Pomianowski, The electrochemical characteristics of the galvanic corrosion of sulfide minerals in short-circuited model galvanic cells, *Hydrometallurgy* 12 (1) (1984) 95–110.
- [138] M. Eghbalnia, D.G. Dixon, Electrochemical study of leached chalcopyrite using solid paraffin-based carbon paste electrodes, *Hydrometallurgy* 110 (1–4) (2011) 1–12.
- [139] S.M.J. Koleini, V. Aghazadeh, A. Sandstrom, Acidic sulphate leaching of chalcopyrite concentrates in presence of pyrite, *Miner. Eng.* 24 (5) (2011) 381–386.
- [140] C.L. Liang, J.L. Xia, Z.Y. Nie, Y. Yang, C.Y. Ma, Effect of sodium chloride on sulfur speciation of chalcopyrite bioleached by the extreme thermophile *Acidianus manzaertsis*, *Bioresour. Technol.* 110 (2012) 462–467.
- [141] C.L. Liang, J.L. Xia, X.J. Zhao, Y. Yang, S.Q. Gong, Z.Y. Nie, C.Y. Ma, L. Zheng, Y.D. Zhao, G.Z. Qiu, Effect of activated carbon on chalcopyrite bioleaching with extreme thermophile *Acidianus manzaertsis*, *Hydrometallurgy* 105 (1–2) (2010) 179–185.
- [142] D.W. Price, G.W. Warren, The influence of silver ion on the electrochemical response of chalcopyrite and other mineral sulfide electrodes in sulfuric-acid, *Hydrometallurgy* 15 (3) (1986) 303–324.
- [143] P.C. Banerjee, B.K. Chakrabarti, S. Bhattacharyya, A. Das, Silver-catalyzed hydrometallurgical extraction of copper from sulfide ores from Indian mines, *Hydrometallurgy* 25 (3) (1990) 349–355.
- [144] J.A. Munoz, D.B. Dreisinger, W.C. Cooper, S.K. Young, Silver-catalyzed bioleaching of low-grade copper ores. Part 1: shake flasks tests, *Hydrometallurgy* 88 (1–4) (2007) 3–18.
- [145] J.A. Munoz, D.B. Dreisinger, W.C. Cooper, S.K. Young, Silver-catalyzed bioleaching of low-grade copper ores. Part 11: stirred tank tests, *Hydrometallurgy* 88 (1–4) (2007) 19–34.
- [146] J.A. Munoz, D.B. Dreisinger, W.C. Cooper, S.K. Young, Silver catalyzed bioleaching of low-grade copper ores. Part III: column reactors, *Hydrometallurgy* 88 (1–4) (2007) 35–51.
- [147] E. Gomez, A. Ballester, M.L. Blazquez, F. Gonzalez, Silver-catalysed bioleaching of a chalcopyrite concentrate with mixed cultures of moderately thermophilic microorganisms, *Hydrometallurgy* 51 (1) (1999) 37–46.
- [148] G. Nazari, D.G. Dixon, D.B. Dreisinger, Enhancing the kinetics of chalcopyrite leaching in the Galvanox (TM) process, *Hydrometallurgy* 105 (3–4) (2011) 251–258.
- [149] G. Nazari, D.G. Dixon, D.B. Dreisinger, The role of galena associated with silver-enhanced pyrite in the kinetics of chalcopyrite leaching during the Galvanox (TM) process, *Hydrometallurgy* 111 (2012) 35–45.
- [150] G.R. Karimi, N.A. Rowson, C.J. Hewitt, Bioleaching of copper via iron oxidation from chalcopyrite at elevated temperatures, *Food Bioprod. Process* 88 (C1) (2010) 21–25.
- [151] J.A. Munoz, M.L. Blazquez, A. Ballester, F. Gonzalez, A study of the bioleaching of a Spanish uranium Ore. 3. Column experiments, *Hydrometallurgy* 38 (1) (1995) 79–97.
- [152] G.M. Gadd, Microbial metal transformations, *J. Microbiol.* 39 (2) (2001) 83–88.
- [153] A. Mishra, N. Pradhan, R.N. Kar, L.B. Sukla, B.K. Mishra, Microbial recovery of uranium using native fungal strains, *Hydrometallurgy* 95 (1–2) (2009) 175–177.
- [154] F. Anjum, H.N. Bhatti, A. Ambreen, Bioleaching of black shale by *Acidithiobacillus ferrooxidans*, *Asian J. Chem.* 21 (7) (2009) 5251–5266.
- [155] A. Bruynesteyn, Bacterial leaching – its impact on the Canadian non-ferrous-metals industry, *CIM Bull.* 76 (851) (1983) 56.
- [156] R.W. Lawrence, A. Bruynesteyn, Biological pre-oxidation to enhance gold and silver recovery from refractory pyritic ores and concentrates, *CIM Bull.* 76 (857) (1983) 107–110.
- [157] F. Acevedo, J.C. Gentina, Process engineering aspects of the bioleaching of copper ores, *Bioprocess Eng.* 4 (5) (1989) 223–229.
- [158] J.A. Brown, V.G. Papangelakis, Interfacial studies of liquid sulphur during aqueous pressure oxidation of nickel sulphide, *Miner. Eng.* 18 (15) (2005) 1378–1385.
- [159] M. Al-Harashsheh, S. Kingman, A. Al-Harashsheh, Ferric chloride leaching of chalcopyrite: synergistic effect of CuCl₂, *Hydrometallurgy* 91 (1–4) (2008) 89–97.
- [160] J.E. Dutrizac, R.J. Macdonald, Effect of Sodium chloride on dissolution of chalcopyrite under simulated dump leaching conditions, *Metall. Trans.* 2 (8) (1971) 2310.
- [161] P.H.M. Kinnunen, J.A. Puhakka, Chloride-promoted leaching of chalcopyrite concentrate by biologically-produced ferric sulfate, *J. Chem. Technol. Biotechnol.* 79 (8) (2004) 830–834.
- [162] Y.F. Cai, X.M. Chen, J.Y. Ding, D.S. Zhou, Leaching mechanism for chalcopyrite in hydrochloric acid, *Hydrometallurgy* 113 (2012) 109–118.
- [163] E. Gomez, A. Ballester, F. Gonzalez, M.L. Blazquez, Leaching capacity of a new extremely thermophilic microorganism, *Sulfolobus rivotincti*, *Hydrometallurgy* 52 (3) (1999) 349–366.
- [164] D.W. Shiers, K.R. Blight, D.E. Ralph, Sodium sulphate and sodium chloride effects on batch culture of iron oxidising bacteria, *Hydrometallurgy* 80 (1–2) (2005) 75–82.
- [165] L. Harahuc, H.M. Lizama, I. Suzuki, Selective inhibition of the oxidation of ferrous iron or sulfur in *Thiobacillus ferrooxidans*, *Appl. Environ. Microbiol.* 66 (3) (2000) 1031–1037.
- [166] H. Deveci, M.A. Jordan, N. Powell, I. Alp, Effect of salinity and acidity on bioleaching activity of mesophilic and extremely thermophilic bacteria, *Trans. Nonfer. Metal Soc.* 18 (3) (2008) 714–721.
- [167] C.S. Gahan, J.E. Sundkvist, A. Sandstrom, A study on the toxic effects of chloride on the biooxidation efficiency of pyrite, *J. Hazard Mater.* 172 (2–3) (2009) 1273–1281.