

Chapter 9

Thermoacidophiles for Bioleaching of Copper



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Abstract Biohydrometallurgy is a modern, steadily developing alternative metal production technology based on the use of microorganisms and their metabolic products, such as ferric iron, sulfuric acid, etc. for the extraction of metals from ores. Microbiological processing of ores and concentrates has economic, technical and, most importantly, environmental advantages over traditional technologies. Heap leaching is successfully used for recovery of copper from a secondary mineral—chalcocite (Cu_2S). However, the main world reserves of copper are found in the form of chalcopyrite (CuFeS_2). Chalcopyrite is the most refractory mineral and undergoes chemical or biological oxidation at a very low rate. One of the most common ways to enhance copper extraction from chalcopyrite is the use of thermo-philic. Besides, the intensity of biooxidation of sulfide minerals depends on the pH, redox potential, $\text{Fe}^{2+}/\text{Fe}^{3+}$ ratio, metals ion concentration and the microorganisms used. It was revealed that the mixed cultures and consortia of moderate thermophilic microorganisms were more efficient and stable in the oxidation of chalcopyrite than pure cultures. From this point of view, developing and optimizing microbial associations for use in commercial copper leaching systems remain an important challenge. In this paper bioleaching of chalcopyrite by pure and mixed cultures of moderate thermophilic bacteria *S. thermosulfidooxidans* and thermotolerant sulfur or iron oxidizing bacteria *L. ferriphilum* CC, as well as the influence of physico-chemical factors on this process have been investigated.

Keywords Bioleaching of chalcopyrite · Kinetics of copper recovery · Moderate thermoacidophiles · Associations of iron- and sulfur-oxidizing bacteria

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9.1 Introduction

Biomining is a modern, steadily developing alternative metal production technology using biohydrometallurgy for the processing of mineral raw materials. Biohydrometallurgy is based on the use of microorganisms and their metabolic products, such as ferric iron, sulfuric acid, etc. for the extraction of metals from ores. Microbiological processing of ores and concentrates has economic, technical and, most importantly, environmental advantages over traditional roasting and autoclave oxidation at high pressures and temperatures (Rawlings 2002; Rawlings et al. 2003).

Biohydrometallurgical processes in the biomining industry are implemented in heap leaching and continuous tank leaching reactors. Leaching has been used to recover copper from ores since ancient times (Ehrlich 1999). However, the development of modern commercial bioleaching processes was associated with the discovery of the first sulfur and iron oxidizing bacteria *Acidithiobacillus ferrooxidans* in the middle of the nineteenth century (Temple and Colmer 1951).

Commercial heap bioleaching was first implemented at the Bingham Kanyon copper mine (Utah, USA) to recover copper from low-grade ores. Subsequently, since 1980, numerous heap bioleaching units of copper have been put into operation in many countries of the world and at the end of the last century, world copper production by biohydrometallurgy reached to 25% (Brombacher et al. 1997).

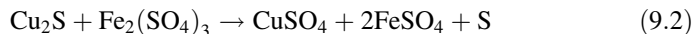
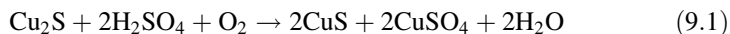
Tank leaching is widely used in the biomining industry for a number of advantages. Tank leaching is a highly controlled process and provides high bioleaching efficiency in terms of rate and recovery of metals. Constant control of aeration, pH and temperature allows optimizing growth and activity of microorganisms functioning in the tanks. In addition, tank leaching leads to long-term selection and domination of those microorganisms that can grow and function more efficiently under tank leaching conditions.

Tank reactors are used for both bioleaching and biooxidation processes. Bioleaching is the dissolution of insoluble metal sulfides into soluble compounds with further extraction of metals from leaching solutions. Tank bioleaching is used to recover base metals such as copper, zinc, and nickel from the corresponding sulfides.

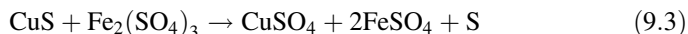
During biooxidation, the target product (metal) passes into a solid phase. Biooxidation is often used to recover gold and silver. The process is based on the oxidation of pyrite and arsenopyrite using microorganisms in order to release gold in the crystal lattice of minerals, followed by its extraction by traditional cyanidation. The first biooxidation unit for the pretreatment of gold ores was commercialized in 1986 by Gencor at the Fairview mine in South Africa (van Aswegen et al. 1991). Due to the minimal ecological impact on the environment and a number of other advantages, tank biooxidation is successfully used to remove iron and arsenic from gold-bearing ores, gradually replacing the indicated physicochemical and pyrometallurgical technologies (Gahan et al. 2012).

Heap leaching of copper is widely used in the biomining industry, but all existing technologies are based on the extraction of copper from a secondary mineral—

chalcocite (Cu_2S) (Olson et al. 2003; Gahan et al. 2012). Chalcocite is readily leached under the action of protons to form covellite (CuS) (Eq. 9.1) and by Fe (III) (Eq. 9.2) formed as a result of bacterial oxidation of Fe (II).



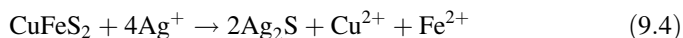
Covellite is also subsequently leached with Fe (III) ions (Eq. 9.3).



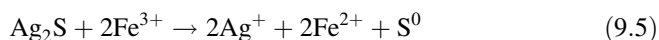
Chalcopyrite (CuFeS_2) is the most abundant sulfide mineral. It is currently estimated that 70% of the world copper reserves now occur in chalcopyrite deposits (Watling 2006). However, chalcopyrite is the most refractory mineral to chemical and biological leaching resulting in low dissolution rates. The main reason for low dissolution rate of chalcopyrite is the formation of passive layer on mineral surface. Reaction products of bioleaching such as elemental sulfur and iron-hydroxide (jarosite) precipitate on the mineral surface and hinder its further dissolution by limiting the flow of bacteria and reaction products to and from the mineral surface (Dopson et al. 2003; Fu et al. 2008; Johnson 2014; Rawlings et al. 1999).

One of the most common solutions to this problem suggested by researchers is to perform bioleaching of chalcopyrite at high temperature using thermophiles. However it worth mentioning that in many cases, instead of extreme thermophiles (70–80 °C) industry has preferred moderate thermophilic microorganisms because they are more resistant to higher pulp densities and higher heavy metal concentrations.

Another alternative for enhancement of chalcopyrite bioleaching is the use of silver as a chemical catalyst. Researchers reported that copper recovery from chalcopyrite was improved through the use of silver as a catalyst in both the presence and the absence of bacteria (Ballester et al. 1990; Gómez et al. 1999; Cancho et al. 2007). It is considered that as a result of a chemical reaction involving an interchange between the silver and the copper and iron from the chalcopyrite lattice silver sulfide is formed on the mineral surface (Eq. 9.4):



Silver sulfide dissolves in the presence of ferric ion and the catalyst is regenerated:



The silver effect is enhanced in the presence of iron and sulfur-oxidizing microorganisms. On the one hand, microorganisms contribute to regeneration of Fe^{3+} and,

on the other hand, oxidize the elemental sulfur layer produced on the chalcopyrite surface preventing chalcopyrite passivation.

9.2 Bioleaching of Sulfide Minerals

9.2.1 Mechanisms of Oxidation of Sulfide Minerals

Currently, there are three main mechanisms in the bioleaching of sulfide minerals (Tributsch 1999) (Fig. 9.1):

Depending on the type of mineral, two different ways of indirect oxidation of minerals are distinguished (Sand et al. 1995, 2001; Schippers and Sand 1999; Suzuki 2001). Metal sulfides, the valence bonds of which are obtained exclusively from metal orbitals, are oxidized with Fe (III) and cannot be attacked by protons (FeS_2 , MoS_2 , and WS_2). The dissolution of these minerals, according to the studies by Steudel (1996) proceeds through the formation of thiosulfate (Fig. 9.2).

Other sulfides, in which the orbitals of metals and sulfur participate in the formation of valence bonds, are soluble in acid and are attacked by both protons and Fe (III) ions (ZnS , CdS , NiS , CoS , CuS , and CuS_2). The dissolution of these

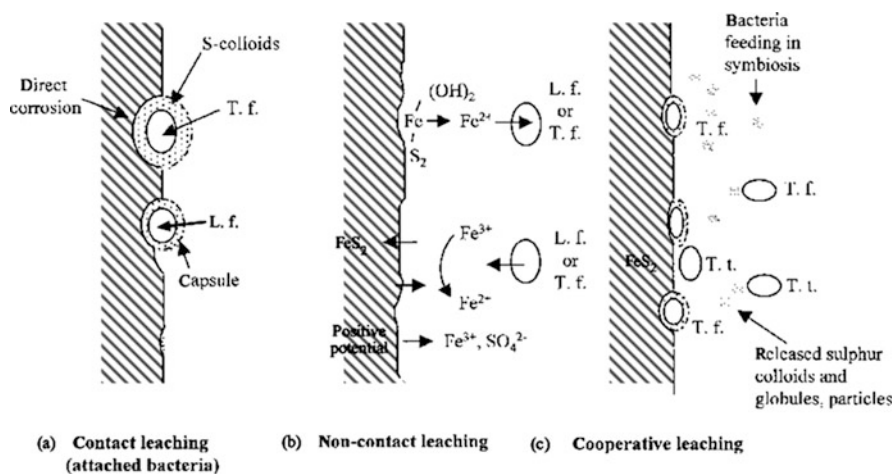


Fig. 9.1 The main mechanisms of bioleaching of sulfide minerals (according to Tributsch 1999): (a) Indirect leaching, when the activity of microorganisms is limited by the regeneration of the leaching agent—Fe (III), (b) Contact leaching, which assumes the attachment of microorganisms to the surface of the mineral, which creates an environment and facilitates the leaching of the mineral through electrochemical dissolution using Fe (III) ions contained in EPS. (c) Cooperative leaching, which assumes cooperation between microorganisms attached to the surface of mineral and free microorganisms in solution. Attached cells, using contact leaching, release and dissolve chemicals that serve as an energetic substrate for free microorganisms

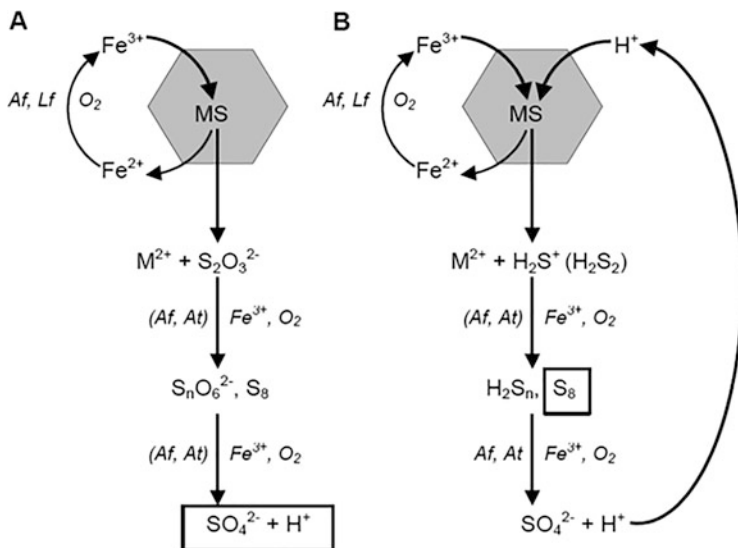
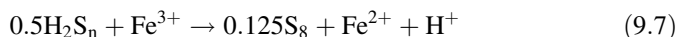
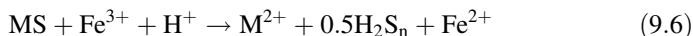


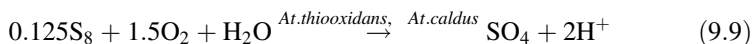
Fig. 9.2 Scheme of thiosulfate (a) and polysulfide (b) mechanisms of indirect oxidation of sulfide minerals (according to Schippers and Sand 1999)

sulfides proceeds by another mechanism—through the formation of polysulfides (Fig. 9.2).

Polysulfide mechanism:



The resulting ferrous iron (Fe^{2+}) and sulfur (S_8) are further oxidized by chemolithotrophic iron and sulfur-oxidizing bacteria (Eqs. 9.8 and 9.9):



Consequently, the role of bacteria in the processes of indirect oxidation of minerals is to supply Fe^{3+} ions (for oxidative attack) and/or protons (for hydrolytic attack).

Contact leaching is based on the attachment of cells to the surface of minerals. The attachment is carried out using extracellular polymer compounds (EPS). Attachment occurs mainly as a result of electrostatic interactions between positively charged cells and a negatively charged mineral (pyrite) at pH 2.0 (Gehrke et al. 1998; Sampson et al. 2000). According to the literature data, the attachment of cells

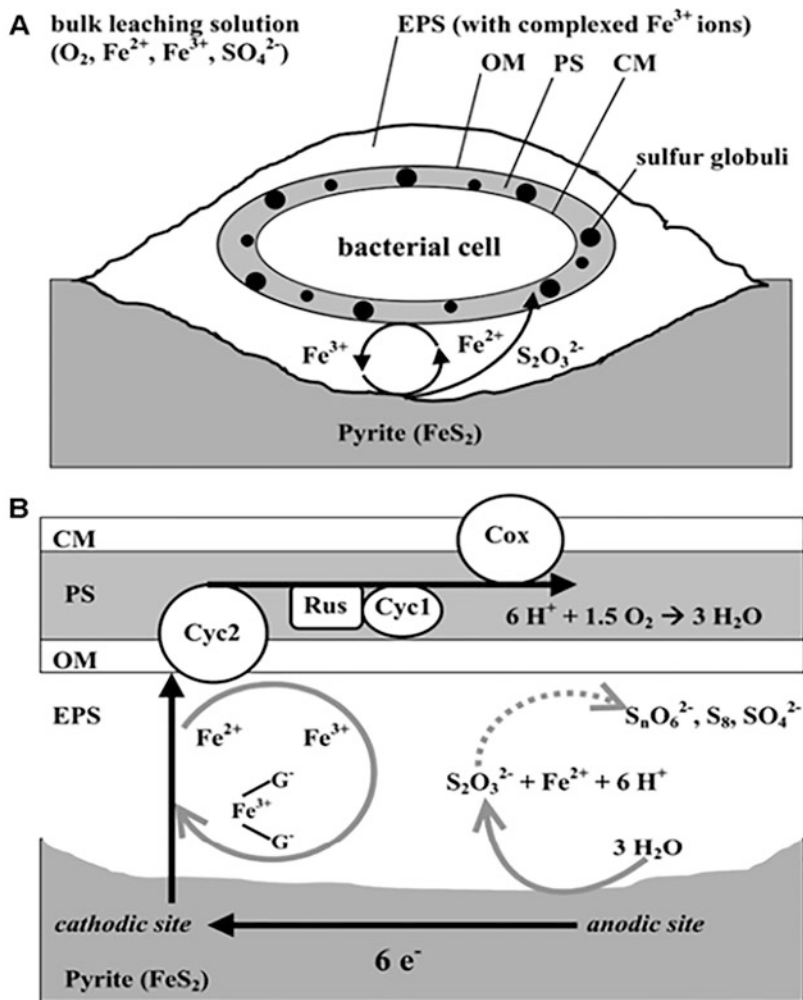


Fig. 9.3 Model of the contact indirect leaching mechanism *At. ferrooxidans* (Rohwerder et al. 2003)

to the mineral does not occur randomly, but preferably to the defective areas of the sulfide mineral (Edwards et al. 1998, 1999; Ohmura et al. 1993; Sanhueza et al. 1999; Gehrke et al. 1998, 2001; Rohwerder et al. 2003). It has been shown that cultures of *At. ferrooxidans* and *Leptospirillum ferrooxidans* have a chemosensory system—chemotaxis, which presents a positive reaction to the Fe (II)/Fe (III) gradient, thiosulfate, etc. (Acuña et al. 1992; Meyer et al. 2002).

It was shown that the rate and intensity of pyrite leaching increase upon direct contact of bacterial cells with the mineral. This is explained by an increase in the concentration of Fe (III) ions in the space between bacterial cells and the surface of pyrite (Fig. 9.3).

This space serves as a reaction medium filled with VPS (Sand et al. 1995; Sand and Gehrke 2006; Rohwerder et al. 2003; Yu et al. 2008, 2011). IPN contain complex ions of Fe (III) in concentrations many times higher than its concentration in the environment. Gehrke et al. (1998) showed the important role of EPS in the pyrite leaching process, since *At. ferrooxidans* cells lacking them are unable to leach pyrite.

9.2.2 *Microorganisms Involved in the Leaching of Sulfide Minerals in Technological Processes*

Bioleaching by microorganisms is widely used for the production of copper and other non-ferrous and precious metals all over the world (Donati and Sand 2007; Rawlings and Johnson 2007). Stirred continuous leaching reactors are characterized by constant parameters—temperature, pH and aeration. The constancy of conditions in the reactors causes a small number of dominant microorganism species. In general, biodiversity in reactors is limited to 2–4 species (Johnson et al. 2008; Rawlings et al. 1999; Sand et al. 1992). At the same time, it has been found that the composition of microbial consortia largely depends on the nature of the minerals and concentrates subjected to leaching (Table 9.1).

In heap leaching, conditions are not the same in terms of irrigation intensity, temperature, pH, aeration, redox potential, nutrient availability, etc., suggesting a wide variety of mineral-oxidizing microorganisms. Therefore, unlike reactors, leach heaps are characterized by significantly greater biodiversity of microorganisms. In this case, the dominant species can vary randomly, as well as depending on the stage of functioning of the heap (Table 9.2).

Thus, the main leaching bacteria belong to the genus *Acidithiobacillus* (= *Thiobacillus*) (Kelly and Wood 2000, 2005). The first representatives of this genus

Table 9.1 Acidophilic bacteria found in tank bioleaching and biooxidation reactors (Rawlings and Johnson 2007)

Concentrates	T (°C)	Procarriotes detected	Source
Zinc/Lead	35–40	<i>L. ferrooxidans</i> , <i>At. thiooxidans</i> , <i>Acidiphilium cryptum</i> , <i>At. ferrooxidans</i>	Goebel and Stackebrandt (1994)
Pyrite/arsenopyrite (gold)	40	<i>L. ferrooxidans</i> , <i>At. thiooxidans</i> , <i>At. ferrooxidans</i>	Dew et al. (1997)
Polymetallic (copper, zinc, and iron sulfides)	45	<i>L. ferriphilum</i> , <i>At. caldus</i> , <i>Sulfobacillus</i> sp., <i>Ferroplasma acidophilum</i>	Okibe et al. (2003)
Pyrite, chalcocopyrite	45	<i>At. caldus</i> , <i>S. thermosulfidooxidans</i> , “ <i>Sulfobacillus montserratensis</i> ”	Dopson and Lindstrom (2004)
Chalcocopyrite	78	(<i>Sulfobolus shibatae</i>), <i>Metalllosphaera</i> , <i>Acidianus infernus</i>	Mikkelsen et al. (2006)

Table 9.2 Acidophilic bacteria found in heap leach reactors

Type of heap, location	Detected microorganisms	Sources
Chalcopyrite (Australia)	<i>At. ferrooxidans</i> , <i>At. thiooxidans</i> , <i>Ac. cryptum</i>	Goebel and Stackebrandt (1994)
Copper sulfide/oxide heap	<i>Acidithiobacillus</i> spp., <i>L. ferrooxidans</i> , <i>Acidiphilium</i> spp., <i>Ferrimicrobium acidiphilum</i>	Bruhn et al. (1999)
Copper sulfide/oxide heap (USA)	<i>Sulfobacillus</i> sp., <i>Firmicutes</i> , <i>F. acidiphilum</i> , <i>Acidisphaera</i> sp., <i>At. ferrooxidans</i> , <i>At. thiooxidans</i> ,	Rawlings and Johnson (2007)
Chalcosite heap (Australia)	<i>L. ferriphilum</i> , <i>At. caldus</i> , <i>Ferroplasma</i> spp.	Hawkes et al. (2006)
Copper heap (Chile)	<i>At. ferrooxidans</i> , <i>L. ferriphilum</i> , <i>F. acidiphilum</i> , <i>Firmicutes</i>	Demergasso et al. (2005)

are extremely acidophilic iron and/or sulfur-oxidizing mesophilic bacteria *At. ferrooxidans*, *Acidithiobacillus thiooxidans*, which with the recently isolated and described moderately thermophilic *Acidithiobacillus caldus* belong to gram-negative γ -proteobacteria. Other leaching proteobacteria belong to the genera *Acidiphilium* and *Leptospirillum* (Hippe 2000; Coram and Rawlings 2002; Sand et al. 1992; Vardanyan and Akopyan 2003). Representatives of the genera *Acidimicrobium*, *Ferromicrobium*, and *Sulfobacillus* are moderately thermophilic gram-positive bacteria (Clark and Norris 1996; Johnson and Roberto 1997; Norris et al. 1996). Leaching archaeobacteria are extreme thermophiles belonging to the genera *Sulfolobus*, *Acidianus*, *Metallosphaera* (Fuchs et al. 1995, 1996; Kurosawa et al. 1998; Norris et al. 2000) and recently isolated mesophilic representatives of the genus *Ferroplasma*—*F. acidiphilum* (Golyshina et al. 2000), *F. acidarmanus* (Edwards et al. 2000).

L. ferrooxidans, *Leptospirillum ferriphilum*, and *Acidimicrobium ferrooxidans* are characterized by narrow specialization- they are able to oxidize only Fe^{2+} , while *At. ferrooxidans* and *Sulfobacillus* spp. bacteria can grow due to the oxidation of reduced compounds of sulfur, Fe^{2+} and other metal ions. *F. acidophilus* (Johnson and Roberto 1997), *Sulfobacillus* spp. bacteria, as well as thermophilic archaea, are mixotrophs and cannot grow autotrophically (Johnson 1998).

Thus, it can be concluded that the most important bacteria in commercial bioleaching processes are iron and sulfur oxidizing bacteria *Acidithiobacillus ferrooxidans*, sulfur oxidizing *Acidithiobacillus thiooxidans* or *Acidithiobacillus caldus* and iron oxidizing *Leptospirillum* spp. bacteria. (Coram and Rawlings 2002; Okibe et al. 2003). Moreover, the dominant bacteria in the bioleaching processes operated at 45–50 °C are *Leptospirillum ferriphilum* and *At. caldus* (Johnson 2014; Johnson et al. 2005; Rawlings and Johnson 2007; Sand et al. 1992; Schippers and Sand 1999). It has been confirmed that the permanent member of the associations, even under mesophilic conditions, is the species *S. thermosulfidooxidans*.

9.2.3 *Factors Affecting the Intensity of Leaching of Sulfide Minerals in Technological Processes*

9.2.3.1 **Influence of the Composition of Microbial Consortia**

The intensity of the process of biooxidation of sulfide minerals depends on the temperature, pH, redox potential, and the nature of the microorganisms used. Among these factors, microorganisms are the most important. A number of studies show that mixed cultures and consortia of microorganisms are more efficient and stable in the oxidation of sulfide minerals than pure cultures (Akcil et al. 2007; Falco et al. 2003; Fu et al. 2008; Baker and Banfield 2003; Johnson 2001).

From this point of view, developing and optimizing microbial consortia for use in commercial leaching systems remains an important challenge. Currently, two different “top-down” and “bottom-up” approaches have been proposed to develop the optimal microbial consortia for tank leaching (Rawlings and Johnson 2007).

The “top-down approach uses a highly diverse mixed culture as an inoculum for leaching sulfide ores. This approach is based on the assumption that a stable and efficient consortium of a limited number of bacteria can be formed during the leaching process, while others disappear. Using this approach, many researchers have succeeded in creating efficient and sustainable consortia for bioleaching at pulp densities no greater than 12% (d’Hugues et al. 2002; Zhou et al. 2009; Watling et al. 2013).

A study of the structures of communities formed during the leaching of chalcopyrite showed that their biodiversity was low (Rawlings and Johnson 2007). At the same time, it was noted that the physiological properties and functions of members of the community were complementary. Thus, it has been shown that this community can include not only iron-oxidizing bacteria (*L. ferriphilum*, *Sulfobacillus acidophilus*, *F. thermoplasma*) and RISCs oxidizing bacteria (*At. caldus*, *S. acidophilus*), but also mixotrophs (*S. acidophilus*, *F. thermoplasma*). Iron-oxidizing bacteria oxidize sulfide minerals using the ferric iron they produce. Sulfur-oxidizing bacteria accelerate the oxidation of minerals by removing the passivating layer of elemental sulfur. Mixotrophs can utilize organic matter contained in exudate or cell lysate, and thus reduce the toxic effect of organic matter on autotrophic bacteria such as *L. ferriphilum*. It is also possible that mixotrophs provide CO₂ for autotrophs. These synergistic interactions between different species increase consortium stability and enhance metal extraction (Johnson 1998; Bacelar-Nicolau and Johnson 1999).

In contrast, a “bottom-up” approach is to create highly efficient, sustainable consortia to leach specific minerals. In such consortia, composite species complement each other in terms of physiological properties, such as the ability to oxidize sulfur and/or iron, to autotrophic or heterotrophic growth, etc. For the creation of such designed consortia, the decisive factors are temperature, pH, concentration of metals, toxic ions, etc. A number of researchers have shown that cultures obtained by this method are most effective in accelerating the oxidation of certain minerals

(Johnson et al. 2008; Akcil et al. 2007; Okibe and Johnson 2004; d'Hugues et al. 2009; Bryan et al. 2011; Mejia et al. 2009).

9.2.4 Bioleaching of Chalcopyrite

Studies have shown that mixed cultures of iron and sulfur oxidizing bacteria *At. ferrooxidans* and *At. thiooxidans* are more effective in leaching chalcopyrite (CuFeS_2) than the corresponding pure cultures. The presence of sulfur-oxidizing bacteria *At. thiooxidans* increases the rate of dissolution of the mineral and the percentage of copper recovery. However, a mixed culture consisting of the moderately thermophilic bacteria *L. ferrooxidans* and *At. caldus* leaches chalcopyrite more efficiently than the mesophilic bacterium *At. ferrooxidans* in pure and mixed cultures (Fu et al. 2008). On the other hand, it has been noted that after 12–16 days of leaching, the dissolution rate of chalcopyrite *At. ferrooxidans* decreases, which coincides with the formation of jarosite as a passivating layer on the surface of the mineral during bioleaching. The use of a mixed culture consisting of *L. ferriphilum* and *At. caldus* leads to a sharp decrease in the pH of the medium as a result of intense sulfur oxidation, which in turn prevents the formation of jarosite and promotes chalcopyrite leaching (Fu et al. 2008). In addition, *L. ferriphilum* and *At. caldus*, being thermophilic bacteria, leach chalcopyrite more intensively than mesophiles, since the rate of oxidation reactions increases with increasing temperature.

It has been found that cultures containing autotrophic and mixotrophic bacterial species are more effective in stimulating bioleaching of chalcopyrite than mixed cultures containing three or four species, such as *At. caldus*, *L. ferriphilum*, *Sulfobacillus* sp. and *Ferroplasma thermophilum* (Wang et al. 2014).

9.2.4.1 Effect of Growth Conditions

It was previously found that the highest rate of oxidation of elemental sulfur and ferrous iron by moderate thermophilic bacteria *S. thermosulfidooxidans* occurred under mixotrophic conditions in the presence of 0.02% yeast extract (Vardanyan et al. 1990, Vardanyan et al. 2015). The data presented in Table 9.3, showed that the amount of iron and copper transferred into the medium under the conditions of mixotrophic growth of *S. thermosulfidooxidans* str. 69 and str. 86, respectively, were 2.9 and 1.2 times more compared with their autotrophic growth. It should be noted that under autotrophic conditions, the Fe^{2+} ion dominated in the medium, and weak growth of bacteria and oxidation of CuFeS_2 were observed. In contrary to autotrophic conditions during the growth of strains on chalcopyrite under mixotrophic conditions in the presence of yeast extract, the Fe^{3+} ion dominated in the medium. Due to the better growth of the strains and oxidation of sulfide sulfur under mixotrophic conditions, a decrease in pH of the medium to pH 1.7 was observed.

Table 9.3 Oxidation of chalcopyrite (CuFeS_2) by str. 86 and str. 69 under autotrophic condition and in the presence of 0.02% yeast extract (Duration 10 days, CuFeS_2 5%, initial pH 2.0, $t=50^\circ$)

Strains	Growth conditions	Metals leached, mg/L			Final pH
		Fe^{3+}	Fe^{2+}	Cu^{2+}	
Uninoculated control		84	840	580	3.2
<i>S. thermosulfidooxidans</i> str.69	Autotrophic	112	1092	920	3.2
	Mixotrophic	2856	644	1380	1.7
<i>S. thermosulfidooxidans</i> str.86	Autotrophic	112	924	900	3.1
	Mixotrophic	1092	168	1200	2.25

Table 9.4 Bioleaching of copper and iron by sulfobacilli depending on concentration of chalcopyrite (Duration—3 days, pH 1.8, $t=50^\circ$)

Bacterial strains	CuFeS_2 , %	Metals bioleached, g/L mg protein	
		Fe_{total}	Cu^{2+}
<i>S. thermosulfidooxidans</i> subsp. asporogenes 41	1	1.08	1.1
	2	2.7	2.7
	3	3.7	4.5
<i>S. thermosulfidooxidans</i> 86	1	6.2	4.8
	2	15.4	10.0
	3	18.1	14.0
<i>S. thermosulfidooxidans</i> 69	1	1.75	2.3
	2	4.37	4.9
	3	6.0	9.1
<i>S. thermosulfidooxidans</i> VKM V-1269	1	1.44	1.2
	2	2.48	2.7
	3	3.6	5.1

9.2.4.2 Effect of Concentration of Substrate

Table 9.4 shows comparative activities of different strains of *S. thermosulfidooxidans* 86 in oxidation of CuFeS_2 depending on its concentration. The studies have shown that the higher the concentration of chalcopyrite the more the amount of copper and iron is leached. It should be noted that at all concentrations tested, strains 86 and 69 showed significantly higher activity of CuFeS_2 oxidation in comparison with strains VKM V1269 and 41. Thus, str. 86 exceeded strains 1269 and 41 by 4.4–6.3 and 3.2–4.2 times for copper and iron, respectively. In case of str. 69 these values were 1.8–1.9 and 1.2–1.7 times for copper and iron, respectively. Thus, in the oxidation of chalcopyrite among the studied strains, the str. 86 shows the highest activity. This fact can probably be explained by the pronounced high ability of the strain to oxidize sulfur.

The studies have shown that the dependence of the iron and copper leaching rates on chalcopyrite concentrations is described by a typical saturation curve (Fig. 9.4).

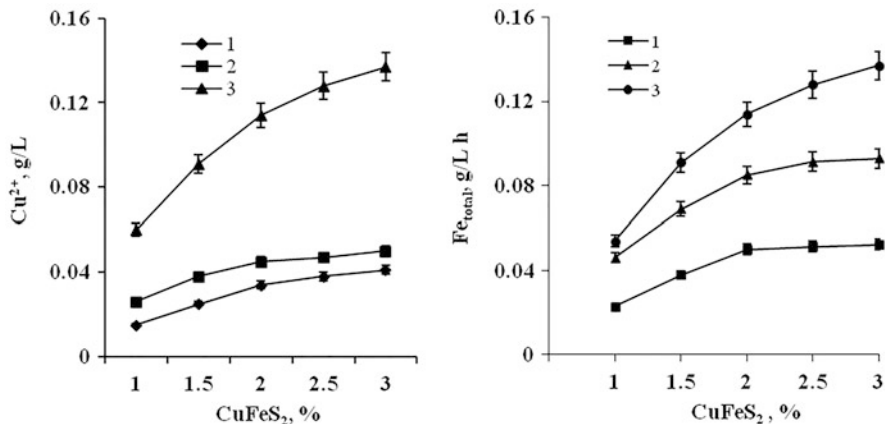


Fig. 9.4 Dependence of copper (a) and iron (b) bioleaching rates on concentration of CuFeS_2 : 1—*S. thermosulfidooxidans* subsp. *asporogenes* str. 41; 2—*S. thermosulfidooxidans* str.69 and 3—str.86

Therefore, by analogy of the kinetics of enzymatic reactions, the bacterial oxidation rates of chalcopyrite can be described by the Michaelis-Menten equation:

$$V = V_{\max}[\text{CuFeS}_2]/[K_m + [\text{CuFeS}_2]] \quad (9.10)$$

The latter in reverse coordinates is known as Lineweaver-Burk the modification:

$$1/V = K_m/V_{\max}[\text{CuFeS}_2] + 1/V_{\max} \quad (9.11)$$

Based on the graph of the dependence of the iron and copper leaching rates on concentration of chalcopyrite in reverse coordinates $1/V$ and $1/S$, the values of K_m and V_{\max} for each strain of *S. thermosulfidooxidans* were determined (Fig. 9.5).

According to the data presented in Table 9.5, str. 86 possess with the lowest value of K_m , consequently the highest affinity to the substrate—1.3% CuFeS_2 . The values of the maximum oxidation rate of chalcopyrite, determined by the leached copper and iron, differ slightly in the studied strains. Nevertheless, relatively high rate of leaching of copper and iron 250 mg/L per hour was observed in str. 86.

9.2.4.3 Effect of pH

The oxidation of chalcopyrite by *S. thermosulfidooxidans* str. 86 was studied in the pH range 1.0–3.0. At the beginning of experiment the increase of pH of the bioleaching solution was observed. According to the mechanism of bioleaching of chalcopyrite the increase in pH occurs due to the consumption of acid during the protonic attack of chalcopyrite (Eq. 9.12):

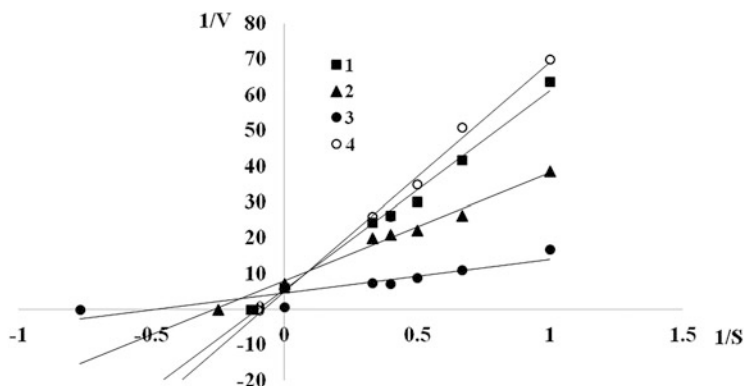
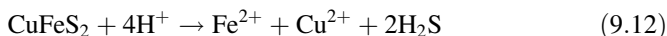


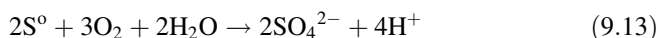
Fig. 9.5 Graphical determination of K_m and V_{max} by the amount of copper leached by *S. thermosulfidooxidans* subsp. *asporogenes* str. 41- (1); str. 69 (2); str. 86 (3) *S. thermosulfidooxidans* VKM-V 1269 according to Lineweaver-Burk

Table 9.5 K_m and V_{max} values for different strains of sulfobacilli during bioleaching of chalcopyrite (pH 1.8)

Bacterial strains	K_m , %		V_{max} , mg/mL h	
	Fe _{total}	Cu ²⁺	Fe _{total}	Cu ²⁺
<i>S. thermosulfidooxidans</i> subsp. <i>asporogenes</i> str. 41	8.0	8.0	0.20	0.17
<i>S. thermosulfidooxidans</i> str. 69	3.6	4.0	0.25	0.14
<i>S. thermosulfidooxidans</i> str. 86	1.8	1.3	0.25	0.2
<i>S. thermosulfidooxidans</i> BKM B-1269	11.8	10.0	0.20	0.17



After 4–5 days pH started to decrease because of the oxidation of elemental sulfur by *S. thermosulfidooxidans* str. 86 (Eq. 9.13):

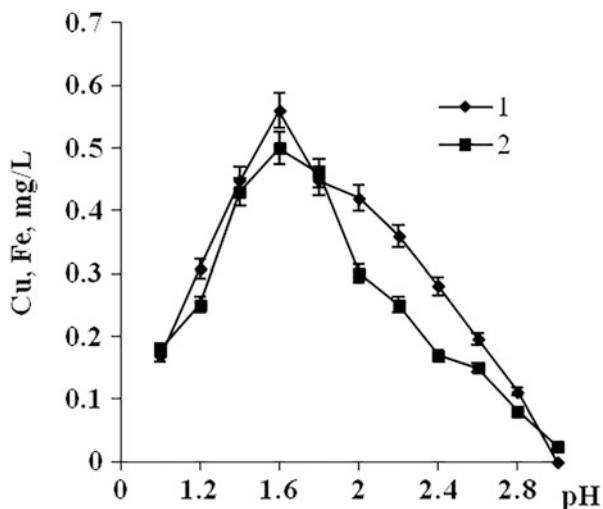


Simultaneously, with pH decrease the extraction of copper and iron from chalcopyrite increased. The data presented in Fig. 9.6 show that the largest amount of copper and iron was leached at pH 1.6. At pH values above 1.8 and below 1.3, the oxidation activity of chalcopyrite decreased, and the process stopped at pH 3.0, the lower pH limit was below 1.0.

9.2.4.4 Effect of Pulp Density

The effect of high concentrations of chalcopyrite on the composition of bacterial consortia during the adaptation of cultures was studied using clone libraries of the

Fig. 9.6 Effect of pH on oxidation of chalcopyrite by 86: 1—Fe leaching, 2—Cu leaching



16S rRNA gene and DGGE analyses. Analyses have shown that with increasing pulp density, the amount of *L. ferriphilum* in the consortium decreases and is not detected at all when the pulp density exceeds 4% (6 or 8%). *At. caldus* and *S. acidophilus* are found in all pulp densities and adaptation stages. The amount of *F. thermoplasma* changes dramatically during adaptation. So, in the initial stages, the bacterium is hardly detected, and at the end it makes up 30% of the consortium. The percentage of *At. caldus* in the consortium at 20% pulp density gradually decreases from 60% at the beginning to 16% at the end of the process. *S. acidophilus* becomes dominant in the middle stages (66%). Despite the fact that the percentage of *F. thermoplasma* in the initial and middle stages was very low, at the end this figure reached 66% (Wang et al. 2014).

In leaching systems functioning under moderately thermophilic conditions, *F. thermoplasma* is the dominant bacterium in the final stages (Zhou et al. 2008; Zhang et al. 2009; Hawkes et al. 2006). It is believed that an increase in pulp density causes high partial pressure, limits the transport of oxygen and carbon dioxide, which leads to inhibition of bacterial growth (Zhou et al. 2009).

An important stage in obtaining highly active cultures of leaching bacteria is their adaptation to high pulp densities. Cultivation under conditions of gradually increasing densities of pulp or metal ions is a well-known method for increasing the bioleaching properties of bacteria and is widely used by many researchers (Astudillo and Acevedo 2008; Zhou et al. 2009; Cameron et al. 2010; Haghshenas et al. 2009; Rawlings 2005).

Studies carried out showed that bioleaching of copper and iron from chalcopyrite enhanced by increasing pulp density (PD) from 2 to 10%. However, at 15% of PD bioleaching of copper and iron by strain *S. thermosulfidooxidans* str. 86 decreased. Maximum extraction of copper and iron by *S. thermosulfidooxidans* str. 86 occurred at 10% of chalcopyrite (Fig. 9.7a, b).

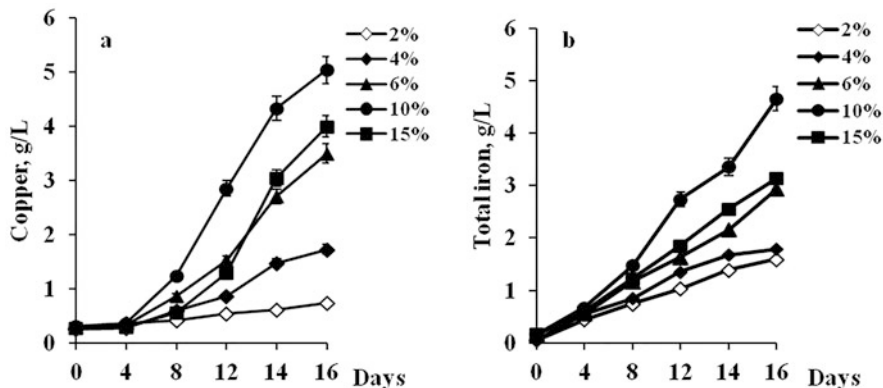
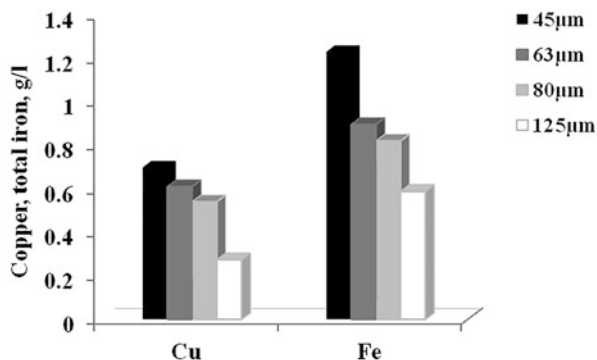


Fig. 9.7 Effect of PD on dynamics of bioleaching of copper (a) and iron (b) by *S. thermosulfidooxidans* str. 86 (pH 1.7; $t=35\text{ }^{\circ}\text{C}$)

Fig. 9.8 Effect of particle size on bioleaching of copper and iron from CuFeS_2 by *S. thermosulfidooxidans* str. 86 (PD—4%, pH 1.8; $t=35\text{ }^{\circ}\text{C}$, 180 rpm/min, duration—16 days)



9.2.4.5 Effect of Particle Size

As shown in Fig. 9.7 the rates of copper and iron dissolution increased with decreasing particle size from +125 μm to size fraction of +45, —63 μm. Maximum yield of copper (0.7 g/L) and total iron (1.23 g/L) reached the size range of +45, —63 μm after 13 days of bioleaching of chalcopyrite by *S. thermosulfidooxidans* str. 86 (Fig. 9.8). The explanation for this increase is that smaller particle size has more surface area, enhancing metal dissolution.

9.2.4.6 Effect of Oxidative-Reductive Potential

It has been shown that Fe^{2+} and Fe (III) ions, depending on the concentration, have different effects on the growth and activity of iron-oxidizing bacteria. Das et al. (1999) report that Fe^{3+} ions at low concentrations stimulate oxygen consumption by bacteria, but at high concentrations they inhibit Fe^{2+} oxidation. A decrease in the rate

of leaching of chalcopyrite at high concentrations of Fe^{3+} has also been reported by other authors (Howard and Crundwell 1999; Third et al. 2002; Hiroyoshi et al. 2007; Petersen and Dixon 2006; Rodriguez et al. 2003).

Córdoba et al. (2008b) studied the effect of iron ions on the dissolution of chalcopyrite at low and high potentials and found that, despite the fact that Fe^{3+} ions were responsible for the oxidation of chalcopyrite, Fe^{2+} played an important role in controlling the formation and deposition of jarosite.

The redox potential (ORP) of the leaching solution depends on the $\text{Fe}^{3+}/\text{Fe}^{2+}$ ratio and is determined by the Nernst equation (Eq. 9.14):

$$E_h = E_h^0 + (R.T/n.F) \times \ln [\text{Fe(III)}/\text{Fe}^{2+}] \quad (9.14)$$

The ORP in leaching systems increases as a result of the activity of iron-oxidizing microorganisms. The increase in ORP promotes the decomposition of minerals such as chalcocite (Cu_2S), covellite (CuS) and pyrite (FeS_2). However, in the case of chalcopyrite, according to some authors, the maximum dissolution rate of the mineral occurs at low ORP (Hiroyoshi et al. 1999, 2000, 2007; Third et al. 2002; Sandström et al. 2005; Córdoba et al. 2008a; Gericke et al. 2010; Ahmadi et al. 2010, 2011).

Thus, ORP is one of the most important environmental parameters affecting the leaching of chalcopyrite and copper concentrates.

The oxidation of chalcopyrite by the mesophilic bacterium *At. ferrooxidans* becomes more difficult with time due to the passivation of the mineral surface. Studies have shown that slowdown of copper leaching is often accompanied by a decrease in the pH of the medium. Based on this, it was assumed that the reason for the decrease in copper leaching was most likely the formation of jarosite. Iron ions, depending on the reaction of the leaching medium, can precipitate in the form of jarosite (Eq. 9.11).



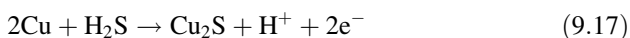
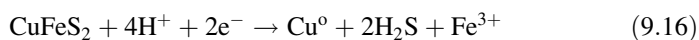
Jarosite forms a passivating layer on the surface of the mineral, which prevents diffusion and hence reduces the rate of chalcopyrite leaching (Stott et al. 2000; Okamoto et al. 2005; Yu et al. 2011).

According to other researchers, the reason for the passivation of chalcopyrite is the high ORP of the leaching medium, which is determined by the ratio of $\text{Fe}^{3+}/\text{Fe}^{2+}$ ions (Third et al. 2002; Gericke et al. 2010). Therefore, one of the approaches to overcome this effect is to maintain a low level of redox potential in the leach solution. The effect of low ORP on chalcopyrite bioleaching is reported in the early studies of Ahonen and Tuovinen (1993). In order to reduce the redox potential of the solution, the authors propose to suppress aeration and thereby inhibit iron oxidation.

It has been shown that the dissolution of copper from chalcopyrite linearly increases at the ORP from 320 to 370 mV. Under these conditions, oxidation of

Fe^{2+} by bacteria is inhibited (Bevilaqua et al. 2014). Córdoba et al. (2008b) studied the effect of ORP on the chemical leaching of chalcopyrite at 68 °C. It was shown that with the ORP of 300–400 mV, almost 90% of copper was leached in 6 days, and with the ORP of 500–600 mV, only 30% in 13 days. Based on the data obtained, the authors concluded that the ORP values from 400 to 450 mV were threshold or limiting, above which the oxidative dissolution of chalcopyrite was gradually slowed down due to the passivating effect (Córdoba et al. 2008a). The release of ferrous iron from chalcopyrite and the precipitation of ferric iron resulted in a low ORP. The same authors report that the ORP rises sharply to 650 mV in bacterial cultures and improves the dissolution of copper, while these conditions do not affect chemical leaching.

Other researchers report that in the presence of 40 mM Fe^{2+} , twice as much copper is leached as when using *At. ferrooxidans*, due to the low ORP in the absence of *At. ferrooxidans* (Hiraishi et al. 2000). The authors proposed a model of chalcopyrite leaching, leading to the formation of chalcocite (Cu_2S), which was significantly rapidly and easily oxidized by oxygen and Fe^{3+} . SO_2 can be used as a chemical reducing agent to suppress ORP.



The leaching rate of chalcopyrite increases with an increase in the ORP of the solution and reaches a maximum value at the optimal ORP. At higher ORPs, the leaching rate decreases.

Similar results were obtained (Third et al. 2000), who studied the effect of stimulating or inhibiting the oxidative activity of iron-oxidizing bacteria in chalcopyrite leaching. The conducted studies allowed them to conclude that the formation of Fe^{3+} as a result of bacterial oxidation of Fe^{2+} inhibited the dissolution of chalcopyrite. The authors proposed to control ORP using oxygen limitation. With controlled ORP, the bacterial oxidation of Fe^{2+} was limited, but at the same time, a sufficient amount of Fe^{3+} was supplied to oxidize chalcopyrite, and as a result, copper extraction increased by 2 times. At the same time, passivation was delayed, but not completely eliminated (Third et al. 2002).

9.2.4.7 Influence of Ferric Iron (Fe^{3+})

Fe^{3+} is a permanent component of chalcopyrite leaching medium. Therefore, the study of the effect of Fe^{3+} ion on its oxidation is of particular importance. Figure 9.9 shows the leaching dynamics of copper and iron during oxidation of chalcopyrite without Fe^{3+} and when Fe^{3+} ion added to the medium.

As can be seen from the data presented in Fig. 9.9, Fe^{3+} ions, at the studied concentrations, stimulate the oxidation of chalcopyrite by str. 86.

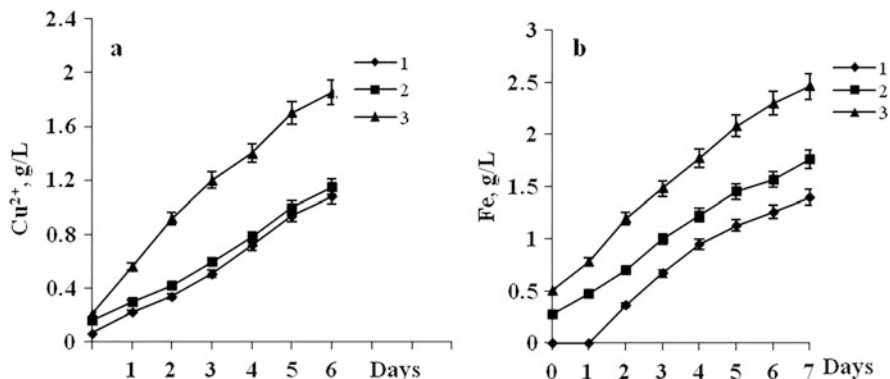


Fig. 9.9 Bioreleasing of copper (a) and iron (b) by *S. thermosulfidooxidans* str.86 during oxidation of chalcopyrite: 1—without addition of Fe³⁺ and in the presence of 2—1.0 g/L Fe³⁺, 3—1.9 g/L Fe³⁺

Thus, Fe³⁺ ion, at concentrations of 1.0 and 1.9 g/L stimulates the oxidation of chalcopyrite in str. 86 by 1.3–1.4 and 1.9 times, respectively. The stimulation effect of Fe³⁺ can be explained by its direct involvement in the oxidation of chalcopyrite, known as the indirect route of its leaching. The Fe³⁺ ion interacts with CuFeS₂ to form Fe²⁺ and elemental sulfur, which are then subjected to bacterial oxidation and react again with chalcopyrite. The stimulating effect of Fe³⁺ has also been confirmed in *At. ferrooxidans* (Keller and Murr 1982; Lizama and Suzuki 1989).

Chalcopyrite is an acid-soluble metal sulfide and consequently is dissolved by both the ferric ions and proton attack (Rawlings et al. 1999; Watling 2006).



Ferric iron oxidizes chalcopyrite to copper and ferrous ions in solution and elemental sulfur (Eq. 9.18). The activity of *L. ferriphilum* CC in mixed cultures resulted in regeneration of oxidative agent—Fe(III). However, the presence of sulfur-oxidizing *At. albertensis* SO-2 in the mixed culture, by which the sulfur is oxidized to sulfuric acid prevents the formation of jarosite on the chalcopyrite surface.

9.2.4.8 Tolerance of Microorganisms to Copper Ion

Acidophilic leaching microorganisms occupy the most metal-rich natural and anthropogenic ecosystems, such as acidic drainage water, tailings and other waste products from concentrators. The study of the resistance and adaptation of these microorganisms to high concentrations of metals is of great scientific and practical interest. This interest is primarily caused by the problem of obtaining strains of

leaching microorganisms resistant to high concentrations of copper for use in biogeotechnological processes.

Studies have shown that when the concentration of copper inside a bacterial cell exceeds the permissible level, the mechanisms of bacterial resistance are activated (Rensing and Grass 2003; Magnani and Solioz 2007). Thus, in gram-negative bacteria, this is an active excretion of copper from the cytoplasm into the periplasmic space by means of p-type ATP-ases localized in the inner membrane (Rensing and Grass 2003). Certain microorganisms can pump or expel copper from the cytoplasm directly into the extracellular space using the resistant nodule division (RND) system. This type of detoxification is well known and described in *E. coli* (Outten et al. 2001). The ability of some bacteria to bind copper in the periplasmic space using copper chaperones has also been reported (Dopson et al. 2003; Puig and Thiele 2002).

The study of the resistance of acidophilic bacteria to copper is limited only to the gram-negative bacterium *At. ferrooxidans*. *At. ferrooxidans* is resistant to high concentrations of copper (up to 800 mM CuSO₄) and other metals (Dew et al. 1999; Orell et al. 2010). *L. ferrooxidans* is able to grow in the presence of 5 mM copper in the medium (Johnson et al. 1992). The resistance of bacteria to copper is very important from the point of view of their application in biotechnological processes, where the concentration of copper ions can vary in the range from 15 to 100 mM CuSO₄ (Watling 2006).

It has been shown that *At. ferrooxidans* ATCC 23270 can function at high copper concentrations due to about 10 genes on the chromosome that are directly related to its copper resistance. They include three genes encoding type p ATPases related to copper transport (copA1Af, copA2Af, and copBAf), three genes related to RND, responsible for the removal of copper from the cell (cusAAf, cusBAf, cusCAf) and two genes, coding periplasmic chaperones for copper (cusFAf and copCAf) (Navarro et al. 2009). Recently, it has been found that the high resistance of some *At. ferrooxidans* strains is due to the presence of additional copper-resistant genes in their genome in the form of genetic islands (GI) (Orellana and Jerez 2011).

Expression of most of these genes was established in *At. ferrooxidans* grown in the presence of high copper concentrations using real-time RT-PCR.

Some of these genes associated with copper resistance were also found in *Leptospirillum* spp. bacteria using metagenomic analyses of nucleotide sequences (Simmons et al. 2008). Two genes were also isolated from *L. ferriphilum* cells, which were responsible for bacterial resistance to arsenic ions. One of them is identical to the gene previously identified in the cells of arsenic-resistant strains *At. caldus* (Tuffin et al. 2006). It has been hypothesized about horizontal gene transfer between leaching and other bacteria, which is an important factor for the transmission of resistance to metals, as well as adaptive and other advantageous properties of these acidophiles (Dopson et al. 2003).

An example of a high tolerance to Fe (III) is the competition between iron-oxidizing bacteria in leaching solutions, which according to Rawlings et al. (1999) flows in favor of *L. ferrooxidans* and *S. themosulfidooxidans* (Vardanyan et al. 1990; Boon et al. 1999). It was shown that Fe (III) ions competitively inhibit the oxidation

Table 9.6 The influence of copper and zinc on oxidation of iron by *S. thermosulfidooxidans* 86 and *L. ferriphilum* CC (for 48 h of cultivation)

<i>S. thermosulfidooxidans</i> 86			<i>L. ferriphilum</i> CC	
Cu, mM	Iron oxidized, g/L, 48 h	Inhibition, %	Iron oxidized, g/L, 42 h	Inhibition, %
0	2.95	0	2.44	0
10	3.53	12.5	1.29	47.1
25	3.5	12.0	0.95	60.9
50	2.44	17.4	0.95	60.9
100	1.62	44.9	0.87	64.3
150	–	–	0.82	66.3
200	0.73	75.3	0.67	72.4
250	0.59	80.1	0.62	74.7

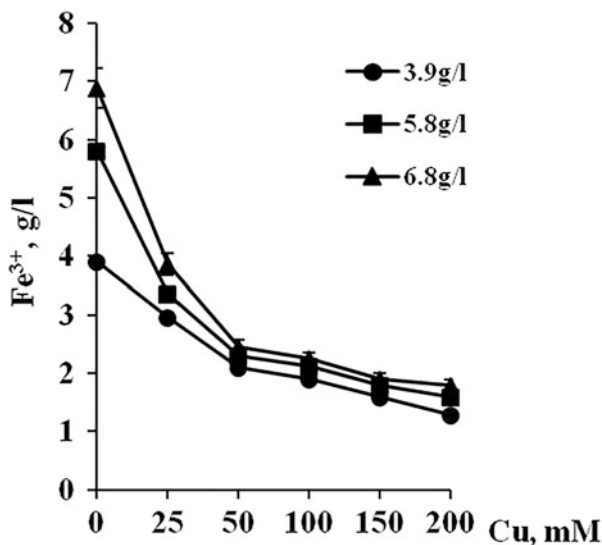
of Fe (II) in *At. ferrooxidans*, *L. ferrooxidans*, and *S. thermosulfidooxidans* (Vardanyan et al. 1990; Boon et al. 1999).

It should be noted that the growth of bacteria in the form of a biofilm significantly increases the resistance of bacteria to metals (Costerton et al. 1995; Harneit et al. 2006; Sand and Gehrke 2006; Sutherland 2001).

The influence of copper on oxidation of iron by *S. thermosulfidooxidans* 86 and *L. ferriphilum* CC was studied in the range of concentration from 10 to 250 mM. It was shown that iron oxidation by *L. ferriphilum* CC is inhibited about 50% in the presence of copper in concentration of 10 mM, while inhibition of iron oxidation by *S. thermosulfidooxidans* 86 reached 45% only at 100 mM copper (Table 9.6). Thus, tolerance of *S. thermosulfidooxidans* 86 to copper was much more higher than that of *L. ferriphilum* CC.

Resistance of microorganisms to metals is of great scientific and practical interest which is caused by the problem of obtaining strains of metal leaching bacteria resistant to high concentrations of metals for application in biogeotechnological processes. In this paper the influence of copper and zinc on the oxidation of iron by *L. ferriphilum* CC and *S. thermosulfidooxidans* 86 depending on substrate concentration was studied. It was shown that the tolerance of *S. thermosulfidooxidans* 86 to copper and zinc was much higher than that of *L. ferriphilum* CC. It was revealed that the increase in the concentration of the substrate led to the decrease in the inhibitory effect of copper and zinc on the oxidation of Fe²⁺ by *L. ferriphilum* CC and *S. thermosulfidooxidans* 86. Thus, the increase of substrate concentration will allow in some ways to overcome the inhibitory effect of copper and zinc. It was established that with the growth of bacteria the decrease of inhibitory effect of metal ions was observed. It is assumed that during their growth bacterial cells form biofilm consisting of extracellular polymeric substances (EPS) and create accordingly a less toxic and more favorable environment for the growth of cells in the presence of copper and other ions (Fig. 9.10).

Fig. 9.10 Effect of Cu on Fe oxidation by *S. thermosulfidooxidans* 86 depending on concentration of Fe^{2+} (pH 1.95; T 35, 200 rpm, 72 h)



9.2.4.9 Oxidation of CuFeS_2 by Mixed Cultures

It is well-known that the most important bacteria in commercial bioleaching processes are iron and sulfur oxidizing bacteria *Acidithiobacillus ferrooxidans*, sulfur oxidizing *Acidithiobacillus thiooxidans* or *Acidithiobacillus caldus* and iron oxidizing *Leptospirillum* spp. bacteria. (Coram and Rawlings 2002; Okibe et al. 2003). Moreover, the dominant bacteria in the bioleaching processes operated at 45–50 °C are *Leptospirillum ferriphilum*, *At. caldus*, and *S. thermosulfidooxidans* (Johnson 2014; Johnson et al. 2005; Rawlings and Johnson 2007; Sand et al. 1992; Schippers and Sand 1999).

It is well-known that in natural biocenoses, there are complex relationships between bacteria, including different forms of reciprocal feeding (Dopson and Lindstrom 1999; Bacelar-Nicolau and Johnson 1999; Johnson 1998; Tuovinen et al. 1994). Taking into consideration the above-mentioned associations of sulfobacilli and other iron and/or sulfur oxidizing bacteria have been developed to significantly increase the activity of sulfobacilli in the oxidation of pyrite and chalcopyrite.

The dynamics of oxidation of chalcopyrite by *S. thermosulfidooxidans* str.86 and thermotolerant sulfur or iron oxidizing bacteria and their association is shown in Fig. 9.11. According to the data presented, the highest oxidation activity of CuFeS_2 showed *S. thermosulfidooxidans* str.86 growing under mixotrophic conditions in the presence of 0.02% yeast extract. The studies have shown that neither iron oxidizing *L. ferrooxidans* str.72, nor sulfur oxidizing bacteria *At. tanzuti* str.5 in monoculture are capable of performing the oxidation of chalcopyrite. However, when *L. ferrooxidans* str. 72 was grown in association with sulfur oxidizing bacteria str. 5 an increase of oxidation of chalcopyrite was observed (Fig. 9.11).

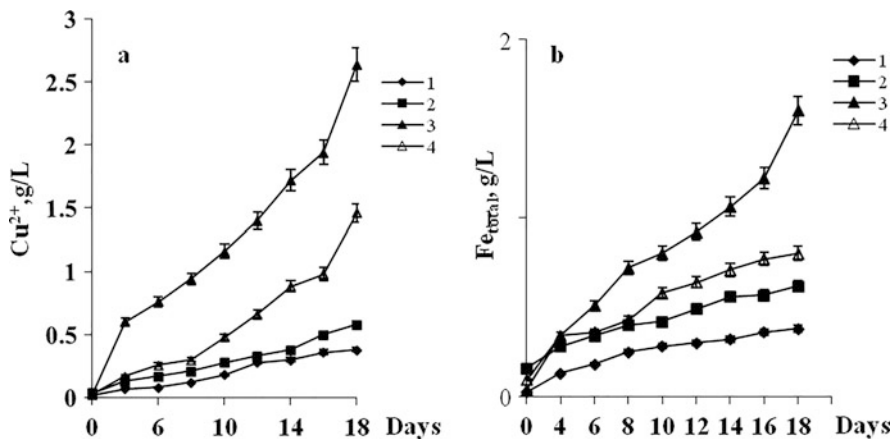


Fig. 9.11 Oxidation of chalcopyrite by: 1—*At. tanzutii* str. 5, 2—*L. ferrooxidans* str.72 3—*S. thermosulidooxidans* str. 86*, 4—*L. ferrooxidans* str.72 + *At. tanzutii* str. 5

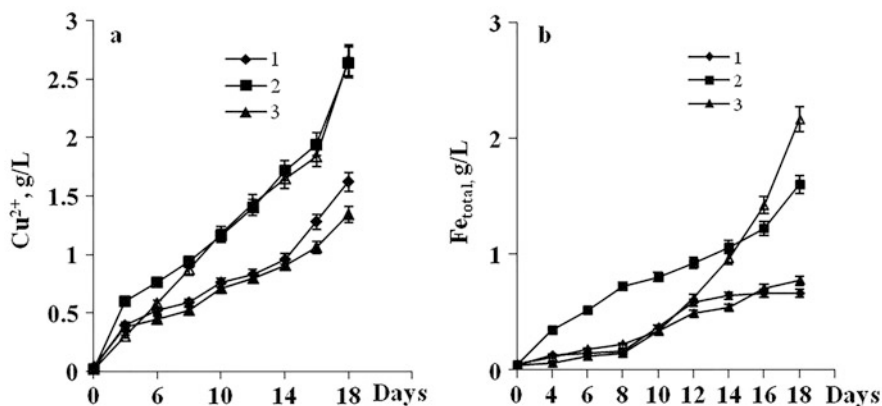


Fig. 9.12 Bioleaching of copper (a) and iron (b) by sulfur and iron oxidizing bacteria during the oxidation of chalcopyrite by: 1—*S. thermosulidooxidans* str. 86, 2—str. 86*, 3—str. 86 + *L. ferrooxidans* str. 72, 4—str. 86 + str. 5

The same poor oxidation of CuFeS_2 was observed during autotrophic growth of *S. thermosulidooxidans* str. 86 (Fig. 9.12). The studies carried out showed that the activity of autotrophically growing str. 86 significantly increased when grew together with *At. tanzutii* str. 5. Thus, for 8 days of cultivation by the amount of leached copper (0.96 g/L), this association reached the efficiency observed by mixotrophically growing str. 86 (0.94 g/L). During 18 days of growth, the mentioned association by the amount of leached iron (2.16 g/L) significantly exceeds str. 86 grown under mixotrophic conditions in the presence of yeast extract (1.6 g/L). It should be noted that no significant oxidation of CuFeS_2 was observed by

S. thermosulfidooxidans growing together with *L. ferrooxidans* str. 72 in the absence of yeast extract with str. 72 (Fig. 9.12).

It can be concluded that the co-cultivation of sulfobacilli with thermotolerant sulfur oxidizing bacteria allows to achieve the efficiency of oxidation of CuFeS_2 without adding an organic carbon source to the medium, which is necessary for the growth of sulfobacilli extract (Vardanyan 1998, 2003; Vardanyan and Vardanyan 2016).

Thus, the use of thermotolerant sulfur-oxidizing bacteria with sulfobacilli allows to perform the oxidation of chalcopyrite without the addition of organic substances by intensity, observed during the growth of moderate thermophiles under mixotrophic conditions in the presence of yeast extract. Syntrophic relationships are thought to arise between the strains studied during chalcopyrite oxidation. Probably, strain 86 of moderately thermophilic bacteria provides sulfur oxidizing bacteria with elemental sulfur and its reduced compounds.

9.3 Conclusion

It has been shown that the dependence of the copper and iron leaching rates on chalcopyrite concentrations is described by a typical saturation curve. Therefore, by analogy of the kinetics of enzymatic reactions, the bacterial oxidation rates of chalcopyrite can be described by the Michaelis-Menten equation: *S. thermosulfidooxidans* str. 86 has shown the lowest K_m value, consequently the highest affinity to the substrate- CuFeS_2 and highest maximal rate of copper leaching. Decrease of particle size leads to the increase of mineral surface area enhancing copper dissolution. Results obtained have shown that ferric iron (Fe^{3+}) stimulates the oxidation of chalcopyrite in moderate thermophilic sulfobacilli that can be explained by direct involvement of Fe^{3+} in the oxidation of chalcopyrite, known as the indirect route of its leaching. Chalcopyrite is an acid-soluble metal sulfide and consequently is dissolved by both the proton attack and ferric iron (Rawlings et al. 1999; Watling 2006). The Fe^{3+} ion interacts with CuFeS_2 to form elemental sulfur and ferrous iron (Fe^{2+}) (Eq. 9.18), which are then subjected to bacterial oxidation and react again with chalcopyrite.

The activity of iron oxidizing bacteria *L. ferriphilum* CC in association resulted in regeneration of oxidizing agent— Fe^{3+} . However, the presence of sulfur-oxidizing *At. tanzuti* in association, by which the sulfur is oxidized to sulfuric acid prevents the formation of passivating sulfur layer and jarosite on the chalcopyrite surface. It can be concluded that the co-cultivation of sulfobacilli with thermotolerant sulfur oxidizing bacteria allows considerably increasing the efficiency of oxidation of CuFeS_2 without adding an organic carbon source to the medium, which is necessary for the growth of sulfobacilli extract (Vardanyan 1998, 2003; Vardanyan and Vardanyan 2016). Thus, the use of thermotolerant sulfur-oxidizing bacteria in association with sulfobacilli allows to perform the oxidation of chalcopyrite under autotrophic conditions by intensity, observed during their growth under mixotrophic

conditions in the presence of 0.02% yeast extract. Syntrophic relationships are thought to arise between the strains studied during chalcopyrite oxidation. Probably, *S. thermosulfidooxidans* str. 86 provides sulfur oxidizing bacteria with elemental sulfur and its reduced compounds.

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