

REVIEW

Evidences of EPS-iron (III) Ions Interactions on Bioleaching Process Mini-review: The Key to Improve Performance

Ellen Cristine Giese

Centro de Tecnologia Mineral, CETEM, Pedro Calmon 900, CEP 21941-908, Rio de Janeiro, RJ, Brazil.

Article history: Received: 20 February 2019; revised: 15 April 2019; accepted: 27 April 2019. Available online: 05 July 2019. DOI: <http://dx.doi.org/10.17807/orbital.v11i3.1389>

Abstract:

Chemolithotrophic microorganisms have been used on commercial extractive bioprocess of valuable metals as cooper, nickel, zinc and gold, from their ore sulfides or concentrates. Bioleaching is a metal solubilization process that involves the production of oxidizing chemicals as iron(III) ions and sulfuric acid from biomining microbes that promotes an attack of mineral matrix releasing the metal ions of interest. In this mini-review, the interfacial interaction between bacterial cells and mineral surface is promoted by biofilm formation and has been described as fundamental to biological ore dissolution. Complexes formed between iron(III) ions and functional groups from microbial extracellular polymeric substances (EPS) have been recently described by mediating the oxidative attack to sulfide minerals promoting metal solubilization. An approach from bacterial adhesion and their interfacial dynamics by EPS complexed iron(III) ions beside sulfide minerals are described.

Keywords: bacterial adhesion; biofilms; bioleaching; extracellular polymeric substances

1. Introduction

Biologically-assisted leaching of metals is a hydrometallurgical process of sulfide minerals dissolution by biological redox reactions that have been extensively studied and described [1, 2, 3, 4]. Bioleaching occurs through the action of microorganisms group capable of convert insoluble metal oxides or sulfides from low-grade ores or concentrates, to soluble water metal sulfates. Thereafter the metal is leached into solution with the advantage of requiring a low initial capital investment and low operating cost [5, 6, 7].

The bioleaching consists in a chemical process that involves the generation of leaching chemicals as iron(III) cations and protons by certain bacteria capable of adhere to mineral surface through biofilm formation [8, 9, 10, 11]. Biomining microbes used in bioleaching processes are mostly acidophilic, autotrophic and chemotrophic bacterial isolates that occur naturally in sulfide minerals. These

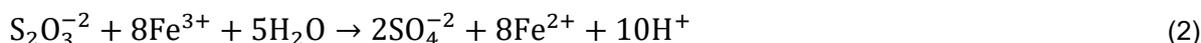
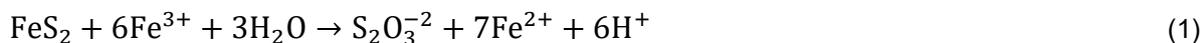
microorganisms getting energy from oxidation of inorganic compounds, as ferrous ion and reduced sulfur species [12, 13].

2. The bioleaching process

The acidophilic iron(II) ion-oxidizing bacterium *Acidithiobacillus ferrooxidans* was the first recognized microbe employed in the processes of extraction of metals from sulfidic ores by bioleaching. *A. ferrooxidans* was considered the dominant microbial population in acid mine drainage due to its great ability to oxidize iron(II) ions and reduced sulfur compounds [14]. In conventional bioleaching processes, the mesophile bacteria *Acidithiobacillus thiooxidans* (S₀-oxidizing and sulfuric acid producer) and *Lepstopirillum ferrooxidans* (iron(II) ion-oxidizing) act synergistically to *A. ferrooxidans*, promoting the most effective solubilization of the metals of interest pertaining to ore sulfides [7, 15].

*Corresponding author. E-mail: egiese@cetem.gov.br

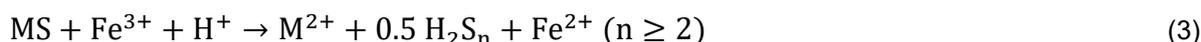
Different mechanisms of bioleaching have been proposed as the mineral dissolution reactions are specific for each metal sulfide [10, 16]:



b) polysulfide mechanism (sphalerite (ZnS), chalcopyrite (CuFeS₂), or galena (PbS)), where metal solubilization is resultant from a combined

a) thiosulfate mechanism (pyrite (FeS₂), molybdenite (MoS₂), and tungstenite (WS₂)), where iron(III) ions oxidize metal sulfides generating thiosulfate as intermediary and main end-product:

attack by iron(III) ions and protons and the elemental sulfur (S₀) as the main intermediate:



In addition, some sulfur-oxidizing bacteria could oxidize S₀ to sulfate and also provide sulfuric acid for a proton attack. The mechanism of solubilization is primarily a chemical process that is current understanding; however, the attachment of microorganisms to the mineral is essential to enhance dissolution and has been best elucidated.

3. Bacterial adhesion to mineral surfaces

Bacteria are often found attached to solid surfaces in nature, especially on rocks and minerals, and its behavior is directly related to microbial activity and survival strategy allowing microorganisms in a nutritionally favorable environment, once adhesion favors the microbes in terms of close proximity to their energy source [17]. Cellular adhesion is a physical-chemical process resulting from the hydrophilic and/or hydrophobic cell surface properties, which is dependent on pH and ionic strength of the solution in which the bacteria are located and also depends of the support surface composition [18].

The bacterial cell wall consists of peptidoglycan macromolecules, a disaccharide of N-acetylglucosamine and N-acetylmuramic acid,

which is present as a linear chain or linked to other substances. At neutral pH, most of bacterial cell walls possess an overall negative charge from carboxyl and amino groups as well as from phosphate groups present in teichoic acids [19]. Considering a bioleaching process, two interfaces are formed: 1) mineral-solution and 2) mineral-bacterial cell; where the bacterial cells living as colloidal particles at the mineral-solution interface [20].

Bacterial adhesion to the mineral surface seems to enhance bioleaching of ore sulfides and can be divided into two steps. Firstly, the presence of extracellular polymeric substances (EPS) mediates the contact between the mineral surface and the cell following by the formation of a biofilm covering the mineral surface with bacterial cells embedded in a continuous layer of EPS. Biofilm formation is the mechanism by which bacterial cells are able to adhere and interact with mineral surfaces [21, 22].

Besides the electrostatic charge conferred by the bacterial cells surface, the electric charge of the biofilm formed on the substrate also depends of chemical characteristics from EPS secreted during the process of bacterial adhesion. EPS are biopolymers in which microorganisms that compose the biofilm are embedded and mainly consist on polysaccharides, proteins and lipids.

EPS can alter the physical and chemical characteristics of surfaces, cells, and consequently affect biofilm formation and cell adhesion [23, 24].

The chemical changes occurred in solution during the ore bioleaching process significantly affect the adhesion forces of bacterial cells [25]. Bacterial adhesion results from the activation of microbial chemotaxis and the number of binding sites accessible to bacteria attachment is also regulated by this biological system. Lactone derivatives via quorum sensing are involved in the process of surface attachment and biofilm formation on mineral sulfides [26].

Microorganisms are eventually attracted to locations electrically charged on mineral surface through chemotactic sensory systems resulting in the formation of permanent anodes and cathodes on the ore surface. The metal dissolution process occurs in the EPS layer formed on the interfacial space between the outer cell membrane and the sulfide ore surface [27, 28].

4. Role of the EPS-iron(III) ions in the dissolution of sulfide minerals

The redox reactions in bioleaching process occur at the interface of mineral, biomining bacterium, and solution [23]. Commonly, leaching bacteria grown attached on the surfaces of ore sulfides and more than 80 % of inoculated cells are adhered to the mineral in the first 24 h. Only some cells may remain in the planktonic state [29, 30, 31].

Biomining bacteria tend to move within the biofilm and thus to the interfacial phase mineral-solution. The mineral surface presents higher concentrations of iron(III) ionic species and also sulfur, facilitating the microbial adhesion by forming EPS-iron(III) ions complexes with the secreted EPS. The EPS-iron(III) ions result from biological re-oxidation of iron(II) ions formed during the chemical oxidation of sulfide mineral. This re-oxidation is mediated by the association of EPS with cytochromes and redox components from bacterial respiratory chain located in the outer membrane [32].

The main contribution of biomining bacteria for bioleaching is to keep the concentration of iron ionic species in its oxidized state, which act as a strong oxidizing agent that promotes the ore

sulfides dissolution as described above. Thus, the formation of EPS-iron(III) ions favors not only the sulfide mineral-bacteria interaction but it is fundamental to comprise a reaction space, in which the dissolution process takes place [9, 23, 33, 34].

The bioconversion process is favored under ionic iron species maintenance in the reaction medium. In this aspect, bioleaching is then favored by the existence of an interfacial bacteria-substrate reaction space, as a formed biofilm, where there is high concentration of oxidizing agent in the form of EPS-iron(III) ions favoring the solubilization of metals through redox reactions.

However, the redox mechanisms involving microbial EPS-iron(III) ions still require elucidation. Once the corresponding interfacial space between the cell membrane and mineral surface distance is equal to at least 2 nm, the reduction of iron(III) ions by electron transfer could occur by tunneling electrons [35, 36]. The electrons are able to cross a barrier of ionic species or molecules, electron donors and recipients, and remain energy unchanged. Another possibility is the smallest stability of glucuronic acid complexed iron(II) ions in comparison to EPS-iron(III) ions [37]. Iron(III) ions could be reduced to iron(II) ionic species and released in solution, and the remaining uronic acids residues would bind to a new iron(III) ionic species striking a balance.

The ability of bacterial EPS to form complexes with ionic species in solution has also been used to explain the solubilization of copper(II) ions from ore sulfides such as chalcopyrite. Yu et al. [38] observed that the presence of copper(II) ions stimulated the production of EPS by *A. ferrooxidans*, and preferentially bind to active sites of these EPS. The proportion of iron(III)/copper(II) linked to EPS decreased from 4:1 to 2:1 when the concentration of copper(II) ions in solution increased from 0.01 to 0.04 mol/L [38]. Figure 1 illustrates the interaction between *A. ferrooxidans* biomining bacteria and copper sulphide mineral surface during a bioleaching process. It can be observed that concomitantly to bacterial conversion of thiosulfate ($S_2O_3^{2-}$) to sulfate (SO_4^{2-}), the EPS-iron(III) ions are reduced to EPS-iron(II) ions with the solubilization of copper(II) ions in solution.

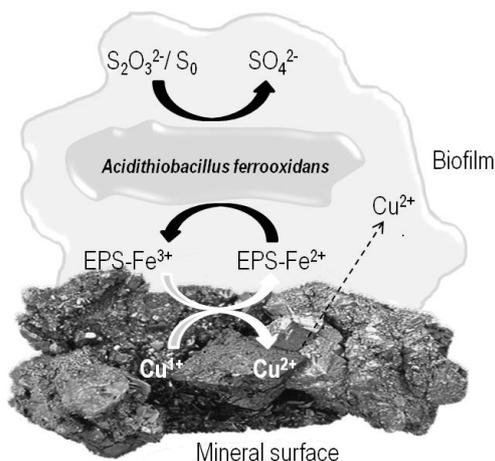


Figure 1. Mechanism of interaction between *Acidithiobacillus ferrooxidans* bacteria and copper sulphide mineral surface during a bioleaching process.

5. Concluding remarks

The biological leaching of mineral sulfides occurs naturally and has been used since ancient times. Due to the progressive exhaustion of mineral deposits, bioleaching has been an important tool for solubilizing metals from mineral substrates with a low grade contents (<0.5%). This biohydrometallurgical process still needs to be improved and its mechanisms understood. The physiological and biochemical parameters of ore-bacteria interaction it has not been well elucidated, in order to clarify the microbial mechanisms of action in bioleaching. However, the studies in situ of microbial growth kinetics and consequent EPS production and biofilm formation during bioleaching process are limited because the experimental reproduction in bench scale is still restricted.

References and Notes

- [1] Olson, G. J.; Kelly, R. M. *Biotechnol. Progress* **1986**, *2*, 1. [\[Crossref\]](#)
- [2] Rawlings, D. E. *Ann. Rev. Microbiol.* **2002**, *56*, 65. [\[Crossref\]](#)
- [3] Olson, G. J.; Brierley, J. A.; Brierley, C. L. *Appl. Microbiol. Biotechnol.* **2003**, *63*, 249. [\[Crossref\]](#)
- [4] Johnson, D. B. *Curr. Opin. Biotechnol.* **2014**, *1*, 24. [\[Crossref\]](#)
- [5] Çelik, H. J. *Ore Dress.* **2008**, *10*, 11. [\[Link\]](#)
- [6] Gehrke, T.; Hallmann, R.; Sand, W. In: *Biohydrometallurgical Processing*. Jerez, C. A.; Vargas, T.; Toledo, H.; Wiertz, J. V., eds. Chile: Elsevier, 1995, chapter 1. [\[Link\]](#)
- [7] Erüst, C.; Akcil, A.; Gahan, C. S.; Tuncuk, A.; Deveci, H. *J. Chem. Technol. Biotechnol.*, **2013**, *88*, 2115. [\[Crossref\]](#)
- [8] Sand, W.; Gehrke, T.; Hallmann, R.; Schippers, A. *Appl. Microbiol. Biotechnol.*, **1995**, *43*, 961. [\[Crossref\]](#)
- [9] Tributsch, H. *Hydrometal.* **2001**, *59*, 177. [\[Crossref\]](#)
- [10] Rohwerder, T.; Gehrke, T.; Kinzler, K.; Sand, W. *Appl. Microbiol. Biotechnol.* **2003**, *63*, 239. [\[Crossref\]](#)
- [11] Vera, M.; Krok, B.; Bellenberg, S.; Sand, W.; Poetsch, A. *Proteom.* **2013**, *13*, 1144. [\[Crossref\]](#)
- [12] Bosecker, K. *FEMS Microbiol. Rev.*, **1997**, *20*, 591. [\[Crossref\]](#)
- [13] Schippers, A. In: *Microbial Processing of Metal Sulfides*. Donatti, E. R.; Sand, W., eds. USA: Springer Link, 2007. [\[Crossref\]](#)
- [14] Sand, W.; Rhode, K.; Sobotke, B.; Zenneck, C. *Appl. Environ. Microbiol.*, **1992**, *58*, 85. [\[Link\]](#)
- [15] Liu, H-L.; Chiu, C-W.; Cheng, Y-C. *Technol. Bioeng.*, **2003**, *83*, 638. [\[Crossref\]](#)
- [16] Schippers, A.; Sand, W. *Appl. Environ. Microbiol.*, **1999**, *65*, 319. [\[Link\]](#)
- [17] Ehrlich, H. L.; Newman, D. K. *Geomicrobiology*, 5th ed., USA: CRC Press, 2009.
- [18] Bos, R.; Van Dermei, H. C.; Busscher, H. J. *FEMS Microbiol. Rev.*, **1999**, *23*, 179. [\[Crossref\]](#)
- [19] Cooper, G. M.; Rausman, R. E. *The Cell: A Molecular Approach*, 6th ed., USA: Sinauer Associates, 2013.
- [20] Vilinska, A.; Rao, K. H. *Sci J.*, **2009**, *2*, 1. [\[Crossref\]](#)
- [21] Roberto, F. F. *Encyclopedia of environmental microbiology*, 2003. [\[Crossref\]](#)
- [22] Siezen, R. J.; Wilson, G. *Microb. Biotechnol.* **2009**, *2*, 297. [\[Crossref\]](#)
- [23] Kinzler, K.; Gehrke, T.; Telegdi, J.; Sand, W. *Hydrometal.* **2003**, *71*, 83. [\[Crossref\]](#)
- [24] Vu, B.; Chen, M.; Crawford, R. J.; Ivanova, E. P. *Molecules* **2009**, *14*, 2535. [\[Crossref\]](#)
- [25] Diao, M.; Taran, E.; Mahler, S.; Nguyen, T. A. H.; Nguyen, A. V. *Coll. Surf. Biointer.* **2014**, *115*, 229. [\[Crossref\]](#)
- [26] Gehrke, T.; Hallmann, R.; Kinzler, K.; Sand, W. *Water Sci. Technol.* **2001**, *43*, 159. [\[Link\]](#)
- [27] Acuña, J.; Rojas, J.; Amaro, A. M.; Toledo, H.; Jerez, C. A. *FEMS Microbiol. Lett.* **1992**, *96*, 37. [\[Crossref\]](#)
- [28] Tan, S. N.; Chen, M. *Hydrometal.* **2012**, *119-120*, 87. [\[Crossref\]](#)
- [29] Bagdigian, R. M.; Meyerson, A. S. *Biotechnol. Bioeng.* **1986**, *28*, 467. [\[Crossref\]](#)
- [30] Gehrke, T.; Telegdi, J.; Thierry, D.; Sand, W. *Appl. Environ. Microbiol.* **1998**, *64*, 2743. [\[Link\]](#)
- [31] Harneit, K.; Göksel, A.; Kock, D.; Klock, J. H.; Gehrke, T.; Sand, W. *Hydrometal.* **2006**, *83*, 245. [\[Crossref\]](#)
- [32] Bellenberg, S.; Leon-Morales, C-F.; Sand, W.; Vera, M. *Hydrometal.* **2012**, *129-130*, 82. [\[Crossref\]](#)
- [33] Sand, W.; Gehrke, T.; Jozsa, P. G.; Schippers, A. *Process Metal.* **1999**, *9*, 27. [\[Crossref\]](#)
- [34] Fowler, T. A.; Holmes, P. R.; Crundwell, F. K. *Appl. Environ. Microbiol.* **1999**, *65*, 2987. [\[Link\]](#)

- [35] Medvedev, D.; Stuchbrukhov, A. A. *J. Theor. Biol.* **2001**, *210*, 237. [\[Crossref\]](#)
- [36] Taylor, E. S.; Lower, S. K. *Appl. Environ. Microbiol.* **2008**, *74*, 309. [\[Crossref\]](#)
- [37] Smith, R. M.; Martell, A. E. NIST critical selected stability constants of metal complexes database. NIST standard reference database 46, ver 8.0. Gaithersburg: Texas A&M University, 2004. [\[Link\]](#)
- [38] Yu, R. I.; Liu, J.; Chen, A.; Zhong, D. L.; Li, Q.; Qin, W. G.; Qiu, G. Z.; Gu, G. H. *Trans. Nonferr. Metals Soc. China* **2013**, *23*, 231. [\[Crossref\]](#)