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Characterization of Bornite (Cu_5FeS_4) Electrodes in the Presence of the Bacterium *Acidithiobacillus ferrooxidans*

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Eletrodos de bornita foram caracterizados na presença e na ausência de *Acidithiobacillus ferrooxidans*, um importante microorganismo envolvido nos processos de lixiviação de metais. A presença da bactéria modificou significativamente a interface mineral/eletrólito, aumentando a taxa de corrosão, como revelado pelas análises interferométricas, AEM, ICP e EIS. A atividade bacteriana sobre o eletrodo de bornita o tornou poroso e conseqüentemente aumentaram sua área e heterogeneidade. Este comportamento foi correlacionado com a evolução dos diagramas de impedância obtidos durante o tempo do experimento. A principal diferença nestes diagramas foi a presença de uma característica indutiva (até 44 h), a qual está relacionada com a ação bacteriana sobre a dissolução do mineral e não com sua adesão. A impedância real total medida na presença da bactéria foi cerca de 10 vezes menor do que na sua ausência, devido a aceleração na dissolução do mineral, uma vez que é mantido o meio oxidante.

Bornite electrodes were characterized in the absence or in the presence of *Acidithiobacillus ferrooxidans,* which is an important microorganism involved in metal bioleaching processes. The presence of the bacterium modified the mineral/electrolyte interface, increasing the corrosion rate, as revealed by interferometric, AEM, ICP and EIS analyses. As a consequence of bacterial activity the electrode became porous, increasing its surface heterogeneity. This behavior was correlated with the evolution of impedance diagrams obtained during the time course of experiments. The main difference in these diagrams was the presence of an inductive feature (up to 44 h), which was related to bacterial action on the mineral dissolution, better than to its adhesion on the bornite. The total real impedance measured in presence of the bacterium was about 10 times lower than in its absence, due to the acceleration of the mineral dissolution, because an oxidant environment was maintained.

Keywords: *Acidithiobacillus ferrooxidans*, electrochemical impedance spectroscopy (EIS), bornite

Introduction

Acidithiobacillus ferrooxidans, formerly *Thiobacillus ferrooxidans*[1] is an acidophilic chemolithoautrophic bacterium capable to utilize either ferrous iron (Fe^{2+}) or reduced sulfur compounds, including mineral sulfides, as the sole energy sources for its growth. Due to its capacity to oxidize metal sulfides, this bacterium is one of the most important microorganism utilized in industrial operations to recover metals, such as copper, uranium and gold.[2] The knowledge of the mechanisms of bacterial dissolution of sulfides has been focused in several copper minerals, in order to improve the efficiency of the bioleaching operations.

The mineral bornite (Cu_5FeS_4) is an important source of copper and its composition can be written as $2(Cu_2S)CuS.FeS$ to show that copper in the mineral occurs in both monovalent and divalent states.[3]

A large variety of experiments has been performed with mostly natural sulfide samples, in the presence of bacteria and chemicals, which also included studies with sulfides as electrodes in electrochemical cells.[4] Kudaikulova *et al.*[5] have shown that at a pH < 3 the redox process on bornite surface (cathodic or anodic decomposition) is due to the oxidation and reduction of copper sulfides (Cu_2S, CuS, Cu_xS where $1 < x < 2$) and copper ion; iron ions dissolve but are not involved in redox reactions. Nevertheless, the

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biological mechanisms and the reactions that come into play during bornite oxidation are still poorly understood.

Recently the electrochemical impedance spectroscopy (EIS) has been utilized in studies dealing with interactions of biological materials and surfaces, such as microbial corrosion and biofilm formation.[6-8] The analysis of EIS data provides information about physical, chemical and biochemical processes occurring at the electrode (sulfide)/ electrolyte (culture medium) interface in the presence or absence of microorganisms.

This work aims to characterize bornite electrodes in the presence of *A. ferrooxidans* in acid medium, using electrochemical impedance spectroscopy (EIS), Auger electron microscopy (AEM), atomic force microscopy (AFM) and interferometric analyses.

Material and Methods

Bacterial strain and growth conditions

Acidithiobacillus ferrooxidans strain LR was used in this work.[9] The culture was grown in mineral salts medium[10] at pH 1.8 plus ferrous sulfate as energy source. The cells for electrochemical analysis and attachment experiments were obtained after growth for 48 h in a shaker (150 rpm and 30 ºC) by successive washing and centrifugation (5000 *g*) to eliminate residual ferric ion from the medium. The washed suspension was further centrifuged at 12000 *g* for 25 min, washed twice in a Milli-Q water of 18 MΩ cm and finally suspended in 10 mL of the mineral salts medium, using pro analysis purity grade chemicals and Milli-Q water. The cell suspension was standardized by the modified Lowry protein determination method.[11]

Mineral samples preparation

Natural research-grade bornite (Cu_5FeS_4) used in this study was obtained from Ward's Natural Science Establishment (Rochester, NY). The sample contained (m/m) 39.7% Cu, 19.7% Fe, 29.4% S. In addition of bornite, X-ray diffraction analysis revealed the presence of quartz (SiO_2) and minor amounts of pyrite (FeS_2), chalcopyrite ($CuFeS_2$), covelite (CuS) and acanthite (Ag_2S). Samples of bornite were cut in pieces of approximately 1 cm^2 using a diamond saw. One face was hand polished through four grit sizes of silicon carbide paper with a final polishing using alumina suspension of 0.3 μm particle size. To eliminate impurities the samples were sonicated with acetone, ethanol and Milli-Q water (15 min each one), and then dried with pure argon and stored in desiccators before using.

Surface analysis

The samples of bornite were individually incubated in 1 mL mineral salts medium containing or not 0.120 mol L^{-1} of ferrous ions and the cell suspension (~ 5 x 10^{10} cells mL^{-1}). Chemical controls without bacterial inoculation were carried out in parallel. The samples were taken out from the solutions after different incubation times and rinsed exhaustively with purified water. This procedure was used prior to surface analyses, which were performed by different techniques, as presented below.

Interferometric analysis was utilized to study the roughness of bornite samples, before and after incubation. The equipment was a ZYGO model GPIxp (Zygo Corporation, Connecticut - USA); with a vertical scan range of 150 μm (with an extended range of 5 mm), a vertical resolution of 0.1 nm, and a repeatability of 0.1%. Interferometry is a traditional technique in which a pattern of light and dark lines (fringes) results from an optical path difference between a measurement and a reference beams. In this system, an interferometer objective is mounted in a precision piezo-scanning device, which moves vertically (in the Z direction) over the sample. Data are collected from a CCD camera and processed by a Pentium® PC controller. Phase relationships for individual components of the white light spectrum in the interferogram are analyzed using a Zygo's patented Frequency Domain Analysis (FDA) (Zygo Metro Pro version 6.5.1). The result is a surface map with ultra high Z resolution, up to 0.1 nm, independent of the objectives magnification. A Physical Electronics Auger Electron Microscopy (AEM) model 670 was utilized to quantify the elements present on the samples surface. Scanning electron microscopy (SEM) (JEOL, T330 A) and Atomic Force Microscopy (AFM) (Nanoscope III Extended Multimode Atomic Force Microscope, Digital Instruments, Santa Barbara, CA, USA) were utilized to observe the adhesion of *A. ferrooxidans*-LR on bornite surface.

Electrochemical measurements

A Tait type electrochemical cell that allows using working electrodes of different sizes and shapes including sheets was used in this work. This cell type eliminates the need of machining or special mechanical preparation of the samples and facilitates microscopic observation after electrochemical studies. In this cell the working electrode (bornite) is fixed at the cell bottom with a Viton O-ring and the other electrodes are fixed at the cell top. The exposed area of the working electrode was 0.28 cm^2. The electrochemical cell was provided with a jacket connected to a thermostatic bath and a recirculation pump to maintain constant the temperature during the test, which was fixed at 30 °C. An $Ag/AgCl/KCl_{sat}$ electrode connected to the solution through a Luggin capillary was used as reference. A Pt wire with large area was used as auxiliary electrode. A Pt disc electrode connected to the reference by a 10 μF capacitor was used to minimize the low frequency noise and the high frequency phase shift.[12] All immersion tests were carried out using 10 mL of aerated solution containing 0.5 g $(NH_4)_2SO_4$, 0.5 g K_2HPO_4, 0.5 g $MgSO_4.7H_20$ in 800 mL of water (pH 1.8 adjusted with H_2SO_4, ionic strength 0.08 mol L^{-1}). In the inoculated electrochemical cell the concentration of the cells of *A. ferrooxidans*-LR was ~ 5 x 10^9 cells mL^{-1}.

Open-circuit potential (OCP) (E_{OC} vs. time) and electrochemical impedance measurements of the bornite electrode were performed using an electrochemical system from EG&G PARC, model 283 and a Frequency Response Analyzer EG&G PARC, model 1025, monitored by a microcomputer using the M398 software. After the attainment of the steady state the electrode was submitted to electrochemical impedance measurement. Impedance spectra were obtained at the OCP by applying a small amplitude sine wave voltage (10 mV rms) in the frequency range from $1x10^5$ to $1x10^{-2}$ Hz at 10 points per decade. All impedance data are given on a geometric area basis of 0.28 cm^2. Impedance diagrams were recorded at different intervals of time (1, 20, 44, 67, 92, 120 h) in the absence and presence of the bacteria in the electrolyte. Considering the small electrode surface exposed to a volume of culture medium with high bacterial concentration the cell growth can be neglected. For all studied conditions E_{OC} values before and after impedance measurements were the same within 2 mV. At the end of each experiment, the solution was withdraw from the cell and filtered for Cu and Fe analysis by inductively coupled plasma emission spectroscopy (ICP).

Results and Discussion

Figure 1 shows the optical micrograph (A), the 2D image (B) and the 3D surface map (C) for bornite after 10 days of bacterial incubation in presence of 0.12 mol L^{-1} Fe^{2+} ions, using an interferometric microscope. The 2D image (B) indicates regions with different depths, which resulted from a preferential attack of the mineral surface, leading to phases with different compositions confirmed by AEM analysis. The analysis of the 3D map provides parameters such as peak-to-valley (PV) distance and average roughness (Ra). PV and Ra values for polished bornite without any attack were typically around 3 μm and 0.200 μm, respectively. After 10 days of immersion in the mineral solution without ferrous ions or bacteria PV and Ra values were, respectively, 6 μm and 0.300 μm. In the presence of Fe^{+2} ions and bacteria, these values increased significantly, reaching a PV = 13.4 μm and Ra = 1.460 μm. As expected, bornite was preferentially corroded in the presence of the bacteria. This result can be rationalized considering that the bacteria can attack the mineral surface directly and also oxidize Fe^{2+} from the solution to Fe^{3+}, which dissolves the sulfide.

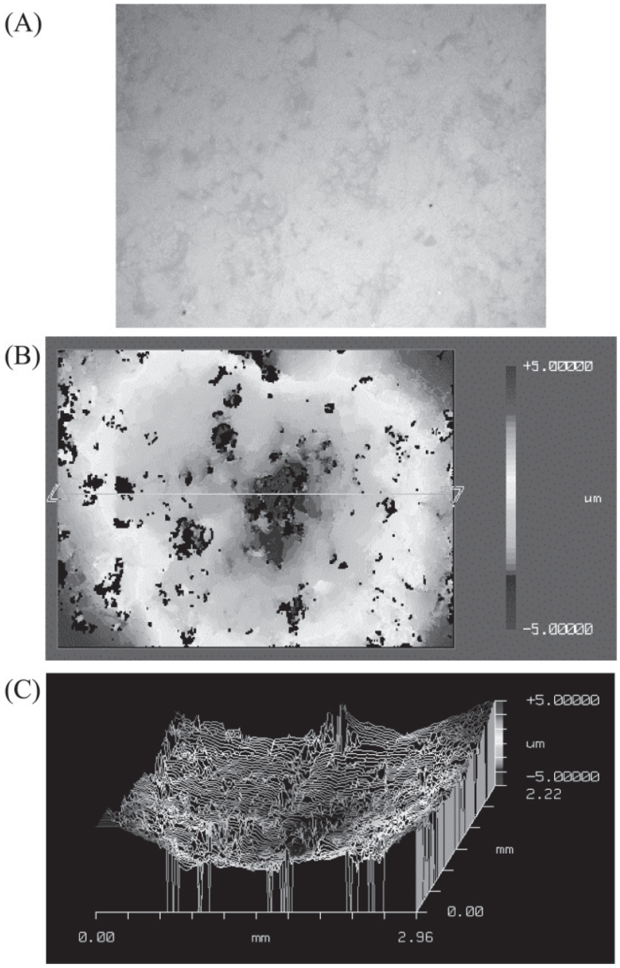

Figure 1. Interferometric images of bornite after 10 days of bacterial incubation in medium with Fe^{2+} as additional energy source. Optical micrograph (A), 2D image (B) and 3D surface map (C). Images obtained after bacterial leaching.

The images obtained with AEM for the original bornite showed two regions of gray color, one dark and other light, revealing some sample heterogeneity. The analysis of these areas revealed different compositions, corresponding to different phases (Figure 2A). In this figure, region 1 contains copper, iron and sulfur as bornite contents, and silver, while iron is absent in the region 2 (Table 1). As

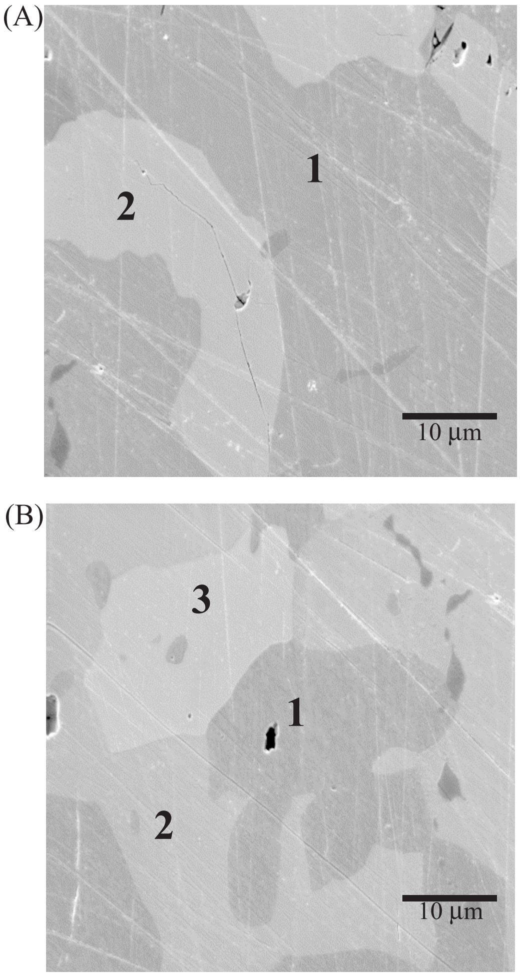

Figure 2. Auger Electron Microscopy (AEM) images of original bornite (A) and after 10 days of incubation in the presence of *A. ferrooxidans*-LR (B). Numbers indicate the analyzed regions.

Table 1. Chemical composition of distinct regions of original bornite and after 10 days of incubation in presence of *A. ferrooxidans*-LR (% m/m)

Sample	Zone	Cu	Fe	S	Ag
Original bornite	1	52.03	23.71	21.36	2.89
	2	71.04	-	19.34	9.61
Bacterial attack	1	48.2	25.71	23.66	2.44
	2	75.89	-	15.14	8.97
	3	72.53	-	27.47	-

mentioned before, the X-ray diffraction analysis (data not shown) of the bornite sample indicated minor amounts of chalcopyrite, pyrite, covelite and acanthite. Then, the absence of iron in region 2 indicates that copper could be also associated with other minerals than bornite, such as chalcopyrite and covelite (CuS). After 10 days of incubation, another gray lighter area was detected (Figure 2B) containing only Cu and S (the secondary covelite) as identified by AEM and confirmed by XRD.[13-15] The elemental composition of each region after bacterial attack is shown in Table 1. Iron was removed from region 1 by chemical and bacterial attack, and this region was transformed in the secondary covelite. Both regions 1 and 2 presented almost the same composition before and after the incubation; however, the area corresponding to region 1 diminished drastically after chemical and bacterial attack, increasing the relative contribution of regions 2 and 3.

It is also interesting to compare the copper/iron ratio in the solution after chemical and bacterial attack to verify the real influence of the bacteria on the mineral dissolution. The results obtained by ICP analysis of the solution after 5 days of incubation (Table 2) indicated a greater mineral dissolution in the presence of the bacteria (almost 10 fold), while the copper/iron ratio was the same in both conditions. There was no evidence of a preferential dissolution of copper or iron. Therefore, the main effect of the presence of bacteria was to accelerate the mineral dissolution, mainly in the region 1, and the secondary covelite formed during this attack, in agreement with interferometric analysis.

Table 2. Copper and iron dissolution of bornite electrode after electrochemical measurement in absence and presence of *A. ferrooxidans*-LR cultures and the corresponding blank

	Cu (mg L^{-1})	Fe (mg L^{-1})
Blank	< 0.05	< 0.10
Absence of *A. ferrooxidans*-LR	3.37	0.77
Presence of *A. ferrooxidans*-LR	39.5	9.10

In a simple way, the bornite composition can be represented as $2(Cu_2S)CuS.FeS$[3] and, in acidic medium and open-circuit condition the iron dissolves chemically and the surface is covered with a layer of covelite (CuS).[5] It is well known that chalcopyrite out of all present phases in the mineral is refractory to acid and bacterial attack.[16,17] On the contrary bornite is easily solubilized,[13,18] probably due to the fact that in its crystalline structure the iron is more exposed,[19] leading to iron dissolution via acid attack. Therefore, the mineral surface is enriched with copper sulfide by chemical attack in experiments with and without the addition of bacteria, explaining the results illustrated in Table 1, where region 1 was mainly attacked. As a consequence of this dissolution, the more active components of the electrode were consumed, increasing the sample porosity and heterogeneity, which can be responsible for the evolution of impedance diagrams obtained during the time course of experiments.

Figure 3 shows a SEM micrograph for bornite after electrochemical experiment in the presence of bacteria, in which it can be seen cells spread all over the surface. The AFM image shows the adhesion of *A. ferrooxidans* on bornite surface (Figure 4) after 3 days of incubation. Both SEM and AFM analysis indicated a bacteria adhesion on the bornite surface even after exhaustive washing before the image was taking out. The bacterial adhesion was also observed even at earlier times (3 h, data not shown). Comparing the surface of the inoculated sample with that surface of a non-inoculated (data not shown), the attack in the presence of bacteria was much more evident. The bacterial attachment on the mineral surface probably facilitates the sulfide oxidation, a very well known process in bioleaching studies, which produces sulfate and decreases the local pH value.[2,20,21]

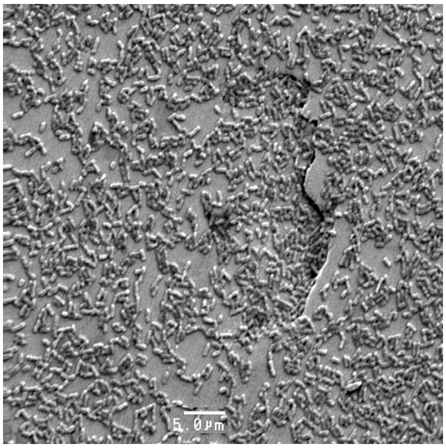

Figure 3. SEM micrograph for bornite electrode after 120 hours immersed into *A. ferrooxidans*-LR containing electrolyte.

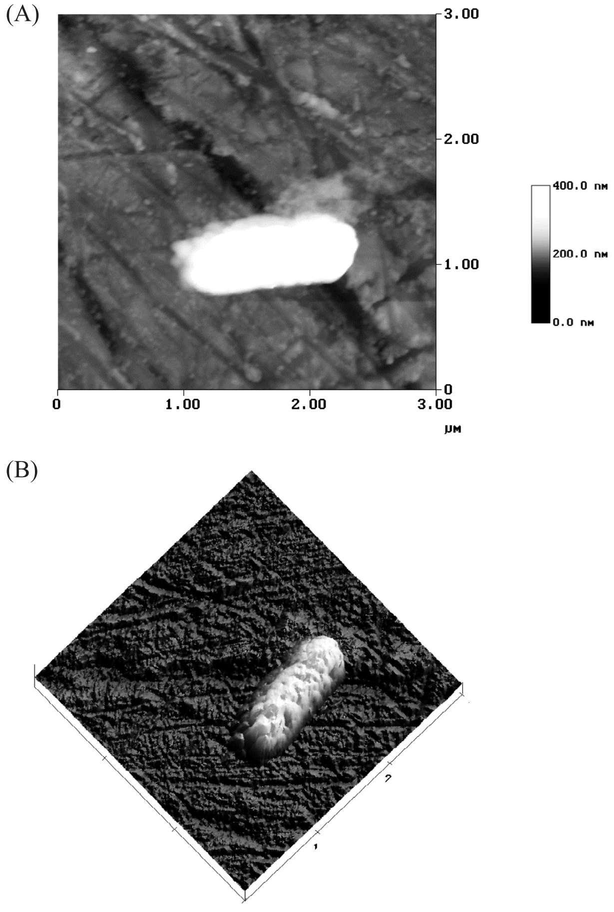

Figure 4. AFM images of bornite surface after 3 days of immersion in the cell suspension. The images are real zoom, (3x3) μm^2 area and 400 nm Z range for (A) and (B).

The bornite (Cu_5FeS_4) can be represented by chalcocite-covelite- ($2(Cu_2S)CuS.FeS$) in which the iron and cuprous ions are oxidized in oxygenated acid medium.[5] Thus, a more refractory phase (secondary covelite) is formed, which is slowly dissolved via acid attack in the presence of oxygen.[13] This acid attack leads to a porous and heterogeneous electrode, which influences the evolution of impedance diagrams with the immersion time. Therefore, even without the bacteria inoculation a corrosion process is occurring at open-circuit potential as a result of anodic and cathodic reactions, which can be illustrated by the global equations:

$$Cu_5FeS_4 \begin{cases} CuS.FeS + 2H^+ \rightarrow CuS + Fe^{3+} + H_2S + e^- & (1) \\ 2Cu_2S + 2H_2S \rightarrow 4CuS + 4\,H^+ + 4e^- & (2) \end{cases}$$

and,

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \qquad (3)$$

As mentioned above the presence of bacteria accelerated the mineral dissolution. The bacteria can act on the mineral surface mainly by two mechanisms: (a) the direct mechanism where the bacteria extract electrons directly from the mineral and these electrons are transferred by the respiratory chain to the oxygen, the final acceptor. Therefore, the electron is not absorbed from an ion present in the electrolyte but from the mineral; (b) the indirect mechanism where the bacteria oxidize ferrous to ferric ions from the solution, which oxidize the mineral, and thus the bacteria act as a mediator recovering the oxidant environment.[22] In the case of bornite both mechanisms operate, and, therefore, the bacteria adhesion is not a necessary condition to sulfur oxidation due to the fact that Fe^{3+} ion also oxidizes the mineral. The secondary phase (covelite) can be mainly dissolved by the direct mechanism. Preferential attack around the adhered bacteria was not observed, once the mineral dissolution is intense even in the early incubation times by Fe^{3+} ions.

Figure 5 shows experimental impedance diagrams for bornite, recorded in the frequency range from 100 kHz to 10 mHz after different times of immersion of the electrode into the electrolyte and in absence of *A. ferrooxidans*-LR. During the experiment time course, it could be observed two capacitive components deviated from an ideal semi-circle. The correspondent Bode diagrams for all immersion periods can be seen in Figure 6. Analyzing the variation of phase angle with frequency, it can be observed two maximum values, the first one between 10 and 100 Hz and the other at about 100 mHz. The phase angle observed at high frequencies increased with incubation time, suggesting that the capacitive component was progressively more noticeable, while no influence of the immersion time in the angle at low frequencies was noted. The increasing in the impedance value with the immersion time was attributed to a decreasing in the surface activity due to the consumption of the more active components of the electrode. It was confirmed by the AEM analysis, which indicated a diminution of the area of region 1 (Cu, Fe, S and Ag) relatively to the area of regions 2 (Cu, S, and Ag) and 3 (Cu and S).

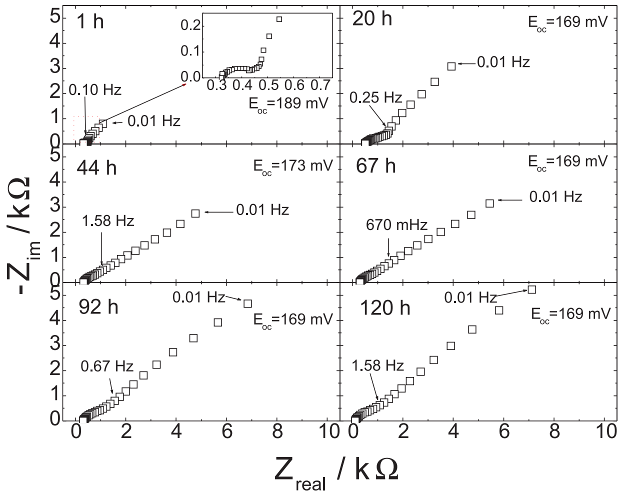

Figure 5. Experimental Nyquist plots of bornite electrode after the attainment of the steady state at different immersion times and in absence of *A. ferrooxidans*-LR. Geometric electrode area = 0.28 cm^2.

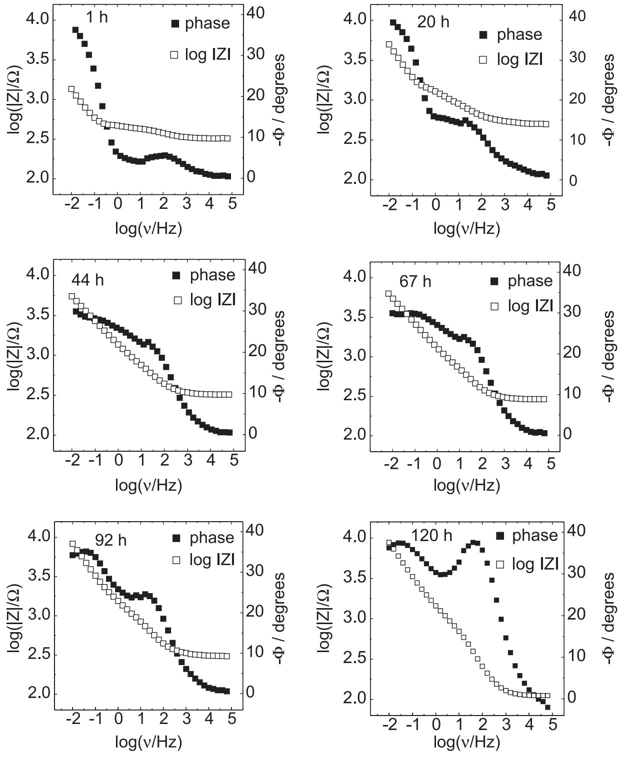

Figure 6. Experimental Bode plots of bornite electrode after the attainment of the steady state at different immersion times and in absence of *A. ferrooxidans*-LR. Geometric electrode area = 0.28 cm^2.

The impedance diagrams can be interpreted like a response of a corrosion process, which is occurring at open-circuit potential as a result of anodic and cathodic reactions, indicated by equations 1, 2 and 3. The first component with a maximum from 10 to 100 Hz can be probably associated with the reduction of oxygen dissolved in the electrolyte, since in a parallel experiment in argon atmosphere (data not shown) this semicircle was modified, suggesting that the reduction of Fe^{3+} to Fe^{2+} ion is the cathodic reaction in absence of oxygen. The second component observed at low frequencies (around 100 mHz) and with higher resistance value, which increased with immersion time, is probably correlated with the oxidation of the cuprous sulfide present at the bornite surface as a consequence of iron and copper dissolution via acid attack,[5] resulting in a heterogeneous electrode. The cuprous ion in the mineral is firstly oxidized to produce a secondary covelite, which is more refractory to acid attack than the natural covelite. The new phase formed is slowly dissolved via acid attack in presence of oxygen,[13] increasing the impedance value. The porosity developed on the electrode surface for longer immersion time, which would hinder further dissolution of the mineral, also contributes to increase the impedance values and to obtain a better capacitive behavior.

The impedance spectra for bornite changed in size and shape with the addition of *A. ferrooxidans*-LR to the electrolyte, mainly at the low frequency range. Figures 7 and 8 show, respectively, experimental Nyquist and Bode plots for bornite recorded in the investigated frequency range, for several immersion times. During the first hours of immersion a capacitive loop followed by an inductive feature and another LF capacitive loop were observed, and after 44 h two capacitive components deviated from an ideal semi-circle were present. The inductive loop increases from 1 to 20 h and decreases at 44 h, tending to disappear for higher immersion times. In a previous work,[13] the XRD of the mineral powder inoculated with *A. ferrooxidans* in a shake flask indicated the absence of the characteristic peaks of bornite after 2 days, while the Fe^{3+} ion concentration increased continuously into the solution

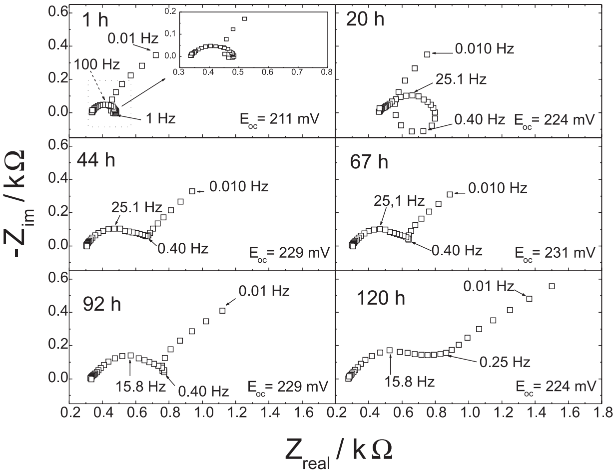

Figure 7. Experimental Nyquist plots of bornite electrode after the attainment of the steady state at different immersion times and in presence of *A. ferrooxidans*-LR. Geometric electrode area = 0.28 cm^2.

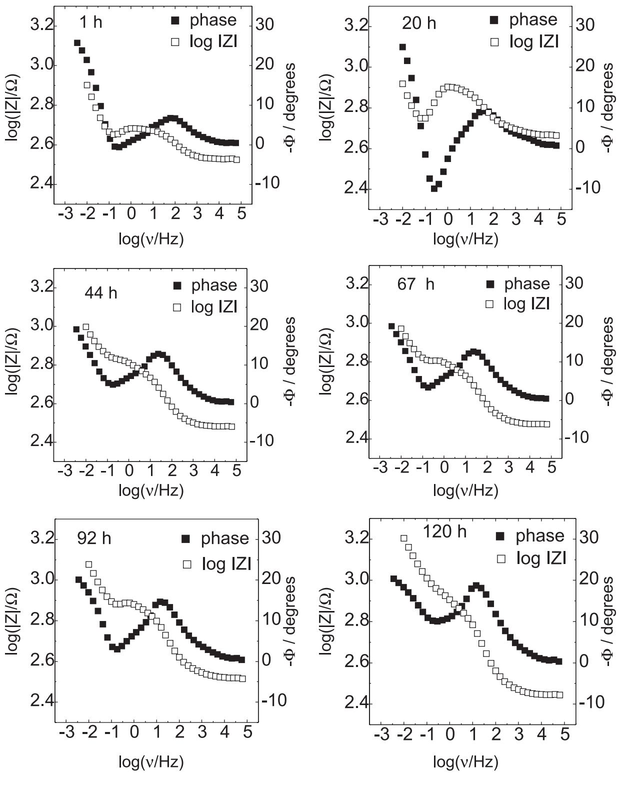

Figure 8. Experimental Bode plots of bornite electrode after the attainment of the steady state at different immersion times and in presence of *A. ferrooxidans*-LR. Geometric electrode area = 0.28 cm^2.

up to 20 days of experiment, and Fe^{2+} ion was not detected. On the contrary, in absence of bacteria Fe^{3+} ion was not detected, and only a low [Fe^{2+}] resulting from the chemical attack to the mineral was found. Therefore, the presence of bacteria is a necessary condition to accelerate the mineral dissolution, which is also assisted by the Fe^{3+} ion, oxidant specie.

In the present work, the massive presence of bacteria accelerated the bornite phase dissolution at the electrode surface, producing more refractory intermediates to dissolution, which can progressively cover the electrode surface, reducing the mineral dissolution rate. Thus, the presence of the inductive loop in the medium frequency range and in the early times indicates that the rapid increase in the oxidation current is impeded at the electrode/electrolyte interface by the inductive current in the opposite direction, which can be a consequence of intermediate species generated by the action of the bacteria. Therefore, the presence of the inductive loop cannot be directly related to the bacteria adhesion, but related to their action on the mineral dissolution, since bacteria attachment was observed even after the inductive loop has disappeared (see Figures 3 and 4). Thus, the increase of impedance with time could be associated with the enrichment of the mineral surface with more refractory phases, for instance the secondary covelite.

At high frequencies, Bode plots also showed an increase in the phase angle with the immersion time, as it was stated in absence of bacteria, suggesting a better capacitive behavior. The total real impedance measured in presence of bacteria was about 10 times lower than in their absence, due to their action on the mineral dissolution rate as stated by ICP analysis (Table 2).

Conclusions

The bacterial action on bornite surface accelerated the mineral dissolution rate via direct and indirect mechanisms, and then both adhered and suspended cells contributed to the corrosion rate. The presence of bacteria increase the corrosion process due to their ability to maintain an oxidant environment rich in Fe^{3+} ion and to increase the dissolution rate of refractory phases comparing to the chemical attack. These facts were reflected in the evolution of impedance diagrams with time, modifying their shape and impedance values. It was evident the major role of bacteria in this process and the proper knowledge of the microorganisms involved constitutes a fundamental key to a possible industrial application in bioleaching. As living beings, microorganisms behave in a very complex way, thus it is necessary to gather as much information as possible about the physiological characteristics of the microorganism and how they can interact with minerals.

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