

Article

Electrochemical Behavior of Ocean Polymetallic Nodules and Low-Grade Nickel Sulfide Ore in *Acidithiobacillus Ferrooxidans*-Coupled Bio-Leaching

Jin-Xing Kang ¹, Ya-Li Feng ^{1,*}, Hao-Ran Li ^{2,*}, Zhu-Wei Du ², Xiang-Yi Deng ¹
and Hong-Jun Wang ¹

¹ School of Civil and Resource Engineering, University of Science and Technology, Beijing 100083, China; kangjx88@126.com (J.-X.K.); wy_3705@163.com (X.-Y.D.); hongjunwang0707@163.com (H.-J.W.)

² State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China; 18811343199@163.com

* Correspondence: ylfeng126@126.com (Y.-L.F.); hrli@ipe.ac.cn (H.-R.L.); Tel.: +86-010-62332467 (Y.-L.F.); +86-010-82627063 (H.-R.L.)

Received: 17 November 2018; Accepted: 16 January 2019; Published: 24 January 2019



Abstract: Efficient extraction of Ni, Co, Cu, and Mn from low-grade and refractory ores is a common technical challenge. The present study proposes an *Acidithiobacillus ferrooxidans*-coupled leaching of Ni, Cu, Co, and Mn from oceanic polymetallic nodules and low-grade nickel sulfide ore, and focuses on the electrochemical behavior of the ores in simulated bio-leaching solutions. In the dissolution of polymetallic nodules, *A. ferrooxidans* facilitates the diffusion of H⁺ and accelerates electron transfer, producing a decrease in charge transfer resistance and promoting the Mn(IV)-preceding reaction. The use of *A. ferrooxidans* is beneficial for lower impedance of sulfur-nickel ore, faster diffusion rate of product layer, and better transformation of the Fe³⁺/Fe²⁺ couple and S⁰/S^{2−} couple. *A. ferrooxidans* increases the potential difference between the nodule cathode and sulfide anode, and increases electron liberation from the sulfide ore. This motivates a significant increase in the average extraction rates of Ni, Co, Cu, and Mn in the bacterial solution. The bio-leaching efficiencies of Ni, Co, Cu, and Mn were as high as 95.4%, 97.8%, 92.2% and 97.3%, respectively, representing improvements of 17.1%, 11.5%, 14.3% and 12.9% relative to that of the germ- and Fe(III)-free acidic 9 K basic system.

Keywords: coupled bio-leaching; electrochemistry behavior; *Acidithiobacillus ferrooxidans*; polymetallic nodules; low-grade nickel sulfide ore

1. Introduction

Due to the danger of completely consuming easily obtainable resources, the extraction of Ni, Cu, Co, and Mn from low-grade and refractory ore has become a focal point [1]. Ocean nodules and low-grade nickel sulfide ore are important, but less tractable, mineral resources of Ni, Cu and Co. In low-grade nickel sulfide ore, nickel minerals occur as fine-particles closely associated with copper, cobalt, iron, and other minerals, resulting in a low recovery of valuable metals in terms of traditional ore enrichment [2]. Ni, Cu, Co, and other metals fill in the interstitial sites of the manganese oxide mineral lattice in ionic form, leading to the necessary destruction of the high valence manganese mineral lattice for the extraction of metals from polymetallic nodules [3]. These metals are present in nodules in the forms of oxides, and in sulfur-nickel ore as sulfides. A critical review of the literature indicates that general inorganic hydrometallurgical routes are uneconomical for extracting these metals from low-grade complex ores [4,5]. Hence, an economical alternative process must be developed, particularly for low-grade polymetallic ores.

Biohydrometallurgy based on microbiological corrosion is an increasingly popular ore extraction technology [6]. In particular, *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) is by far the most widely used bioleaching microorganisms in ore leaching, including for Ni, Cu, Au, V, and Mn ores [7–9]. Additionally, a corrosion cell is the most common method in manganese oxide minerals and sulfide minerals for extracting valuable metals [10]. Many previous research studies have focused on the pyrite-pyrolusite coupled bio-leaching system. However, the *A. ferrooxidans* corrosion system that uses the MnO_2 of polymetallic ore as the electron acceptor and low-grade sulfur-nickel ore as the electron donor has rarely been reported.

The ore *A. ferrooxidans*-leaching is a complex electrochemical corrosion process, related to multiple migrations of substances between phase and interface [11]. As is well known, since its discovery, the mechanism of *A. ferrooxidans* leaching has been proposed to be either a direct or an indirect reaction [12]. In the indirect mechanism of *A. ferrooxidans*, germs catalyze the circulation of the $\text{Fe}^{3+}/\text{Fe}^{2+}$ electron pair for promoting ore leaching [13]. In addition, the diversity of the electron acceptor directly affects the *A. ferrooxidans* bio-leaching [14]. As *A. ferrooxidans* has varying degrees of influence on the electrode process of nickel sulfide or manganese oxide dissolution under different conditions, it is particularly important to study the relationship between its dissolution system and electrode electrochemical behavior [15].

When the corrosion mechanism is studied for coupled bio-leaching of Mn and S-bearing ores, both ores should be evaluated for acid and galvanic corrosion, and dissolution under the influence of Fe(III) or *A. ferrooxidans*. Understanding the roles of Fe(III) and *A. ferrooxidans* needs to employ acid culture, Fe(III)-containing acid culture, *A. ferrooxidans*-containing acid culture, and bacteria with Fe(III) culture. However, most efforts are using *A. ferrooxidans* and Fe(III) mixed culture as the bio-leaching system, and the function analysis of the single action of bacteria or Fe(III) on ores is considerably less-researched.

This work compared the extraction efficiency of Ni, Cu, Co, and Mn from polymetallic nodule and low-grade nickel sulfide ore by *A. ferrooxidans*-coupled and *A. ferrooxidans*-free leaching. The electrochemical behavior and electrode corrosion of the main metal minerals in biotic and abiotic electrolysis solutions were performed using multiple electrochemical methods. The reaction mechanism of the polymetallic nodule cathode and low-grade sulfur-nickel ore anode were discussed in *A. ferrooxidans* and the abiotic sulfuric acid system. The purpose of the present study is to offer a new approach to provide a theoretical reference for the comprehensive development and utilization of low-grade and intractable polymetallic ore resources.

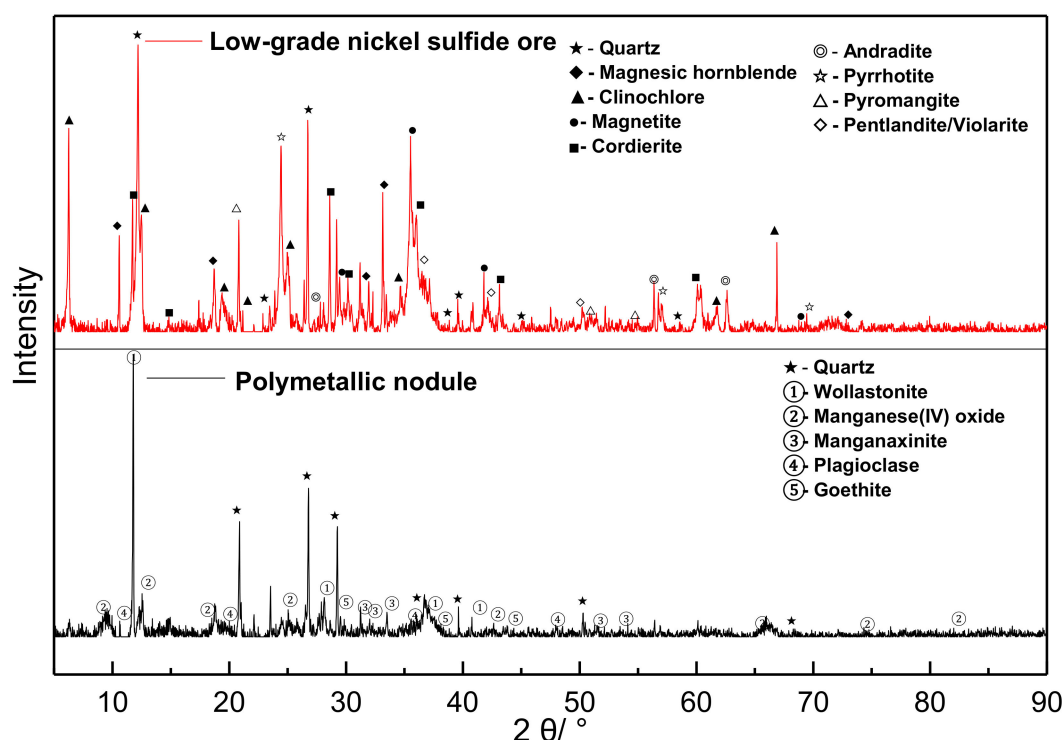
2. Experimental

2.1. Materials and Bacteria Culture

The polymetallic nodule was obtained from the China Pioneer Area of the Eastern Pacific Ocean. The low-grade nickel sulfide ore was collected from Jinchuan, Gansu, China. The nodule and sulfide ore were air-dried, crushed, and ground to 74 μm in the laboratory for the experiments, with the specific surface of 11.07 m^2/g and 5.43 m^2/g (which was measured by nitrogen adsorption method), respectively. The main chemical compositions of the nodule and nickel ores used in this work were determined by chemical element analysis and listed in Table 1. Figure 1 shows the XRD patterns of nodule and sulfide. Manganese, present in the form of high oxide in the polymetallic nodule, and nickel, copper, and cobalt ions filled in the lattice gap of the ferromanganese mineral [16]. The main minerals in the ore include violarite, pentlandite, chalcopyrite, pyrrhotite, and gangue minerals, including augite and chlorite [17]. All other reagents were of analytical grade.

Table 1. Chemical composition of the polymetallic nodule and low-grade nickel sulfide ore (wt.%).

Polymetallic Nodule			Low-Grade Nickel Sulfide Ore		
Ni	Cu	Co	Ni	Cu	Co
1.65	1.40	0.204	0.86	0.57	0.01
Mn	T _{Fe}	S	Mn	T _{Fe}	S
21.84	10.46	0.42	0.27	9.67	7.18
SiO ₂	Na ₂ O	K ₂ O	SiO ₂	MgO	K ₂ O
21.43	0.18	3.20	39.23	32.43	0.42
Al ₂ O ₃	CaO	TiO ₂	Al ₂ O ₃	CaO	TiO ₂
6.42	3.20	1.05	6.12	4.34	0.42

**Figure 1.** XRD pattern of the polymetallic nodule and low-grade nickel sulfide ore.

A. ferrooxidans was obtained from the Environmental Biological Science and Technology Research Center, Institute of Geochemistry, Chinese Academy of Science. The abundance of *A. ferrooxidans* species was determined by sampling bacteria cultivated in 9 K basic medium (g/L) [18]: (NH₄)₂SO₄ 0.15, MgSO₄·7H₂O 0.05, K₂HPO₄ 0.05, Ca(NO₃)₂ 0.01. The growth culture was 9 K medium supplemented with 44.5 g/L FeSO₄·7H₂O to 9 K basic medium. The leaching and electrolytic solutions included a control, Fe(III), *A. ferrooxidans*, and an *A. ferrooxidans* + Fe(III) solution. The control solution was sterile and iron ion-free 9 K nutrient solution. A selected mass of Fe₂(SO₄)₃ dissolved in a sterile 9 K basic solution constituted the Fe(III) solution. The *A. ferrooxidans* solution was bacteria suspended in 9 K solution without iron ion, from which *A. ferrooxidans* was separated by a centrifuge at 12,000 rpm for 20 min from post-log growth culture. The *A. ferrooxidans* + Fe(III) solution was a combination of ferric ion and *A. ferrooxidans* solution. A solution of 20% H₂SO₄ was used to adjust the pH of the solution.

2.2. Coupled Leaching Procedure

Polymetallic nodules and low-grade nickel sulfide ore were mixed in a setting molar ratio of sulfur and manganese (n_S/n_{Mn}). A volume of configured biotic and abiotic solution was employed for

a selected pulp mass fraction. Subsequently, leaching experiments were conducted in a 250-mL conical flask placed in a shaking table at a temperature of 30 °C. Then, 150 mL of solution (*A. ferrooxidans*, *A. ferrooxidans* + Fe(III), Fe(III), or control) was used for each test, and four groups were tested in parallel at the agitation rate of 140 r/min. After an established time, the slurry was filtered and the residue was washed with dilute sulfuric acid, then the leached residue and filtrate were taken for analysis of the elemental content. The mass balances error control was less than 2%. The pH of the leaching medium was kept constant by adjusting with dilute sulfuric acid. The tests were performed under anaerobic atmosphere in closed containers. Using nitrogen obtained the anoxic atmosphere.

Leaching efficiency was calculated based on the amount of leached metal in the residue with respect to the original input quantity. Using UV (UV-1750, SHIMADZU, Tokyo, Japan,) spectrophotometric methods of dimethylglyoxime, 1-nitroso-2-naphthol, sodium diethyldithiocarbamatetrihydrate, periodate potassium, and phenanthroline determined the leached solution concentration of nickel, cobalt, copper, manganese, and Fe(III)/Fe(II) [19]. Mineralogical analysis of polymetallic nodule, low-grade nickel sulfide ore, and residues were performed using X-ray diffraction analysis (XRD, Rigaku, Tokyo, Japan, D/max 2550 VB+; Cu/K α radiation, 40 kV/250 mA).

2.3. Electrode and Electrochemical Measurements

Electrochemical evaluations were performed in a typical electrochemical cell, with three electrodes on a CHI660D microcomputer-based electrochemical system. A Pt plate (1 cm²) electrode was used as an auxiliary electrode and a saturated calomel electrode (SCE) was used as a reference (0.24 V vs. standard hydrogen electrode (SHE)). The preparation of working electrodes deposited a thin layer of mineral ores ink over a Pt disk (0.1 cm²) of the platinum electrode. The mineral grain ink was respectively prepared by mixing the polymetallic nodule or low-grade nickel sulfide ore (80 wt.%), polyvinylidene fluoride (10 wt.%), and acetylene blank (10 wt.%) in N-methyl-2-pyrrolidone [20]. The electrodes dried up at 80 °C. The three electrodes were immersed in the biotic or abiotic sulfuric acid leaching medium for 20 min and the scan was activated. The simulated bath solutions reduced the influence of dissolved oxygen by purging nitrogen. The cyclic voltammetry measurements of ocean polymetallic nodules and low-grade nickel sulfide ore electrodes were performed by sweeping from −0.6 V (positive-going potential scan) to 1.5 V (negative-going potential scan), and back to the beginning potential and by sweeping from 1.2 V (negative-going potential scan) to −0.8 V (positive-going potential scan), and back to the beginning potential with a sweep rate of 50 mV·s^{−1}, respectively. The impedance spectra were obtained at the open circuit potential (OPC) by applying a sine wave voltage amplitude of 5 mV in the frequency range of 0.01–10⁵ Hz. The data were displayed as Nyquist plots and analyzed using Zview2 software. Potentiodynamic polarization curves were generated between −0.8 and 1.0 V with a scanning rate of 5 mV·s^{−1}. The chronoamperometric plots were obtained at the polarization potential of 1.0 V. The Electrochemical measurements were performed at 30 °C by connecting a circulating thermostatically controlled water loop. Refer to SCE for all potentials shown in the paper.

3. Results and Discussion

3.1. *A. Ferrooxidans*-Coupled Leaching Experiment

3.1.1. Effect of Leaching System on Extraction of Ni, Co, Cu, and Mn

Figure 2 shows the leaching efficiency of Ni, Co, Cu and Mn over time in different initial leaching solutions, indicating an increase in Ni, Co, Cu and Mn leaching efficiency from the ores in the presence of *A. ferrooxidans*. The leaching efficiency of coupled leaching is much better than that of leaching alone, suggesting that the coupled leaching forms a corrosion galvanic cell leading to a remarkable improvement of the leaching efficiency. Figure 3 presents the XRD pattern of residues in different leaching systems, illustrating no new phase in the *A. ferrooxidans* system relative to the system without

it. Nickel, manganese, copper, and cobalt metals are nearly dissolved out for processing 96 h in *A. ferrooxidans*, *A. ferrooxidans* + Fe(III), and Fe(III)solutions, but not the control acidic 9 K basic solution. The extraction characteristics of cobalt are similar to those of manganese, while copper is comparable to nickel, suggesting that the digestion of Mn and Co can be attributed to the polymetallic nodule, while Ni and Cu are released from polymetallic nodule and low-grade nickel sulfide ore. Likewise, the leaching efficiency of Ni, Co, Cu, and Mn linearly depended on the time in each solution for the incipient 48 h. The experiments below focused on the 48 h average leaching rate of nickel and manganese and the 168 h extraction of nickel and manganese.

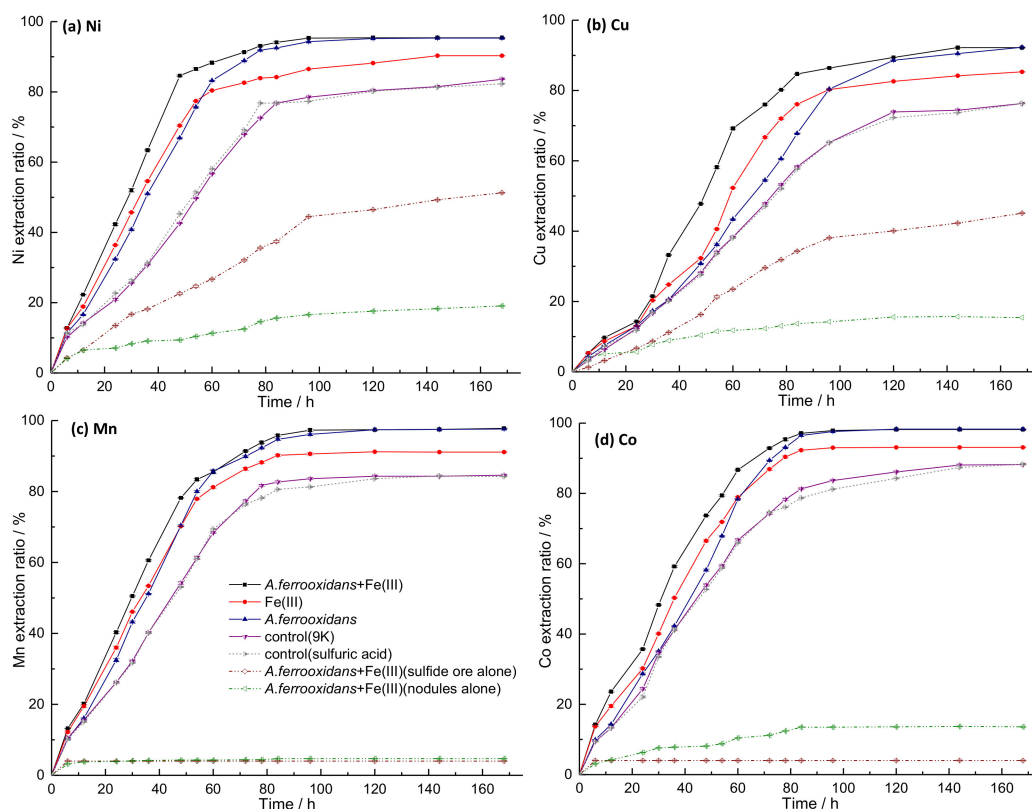


Figure 2. Effect of initial leaching system on major elements leaching ((a) Ni, (b) Cu, (c) Mn, (d) Co; 30 °C; pH 1.8; mole ratio of $n_{(S)}/n_{(Mn)}$ 0.67:1; shaking speed 140 r/min; pulp mass fraction 8%; initial *A. ferrooxidans* amount 1.0×10^8 /mL; Fe(III) concentration 1.0 g/L).

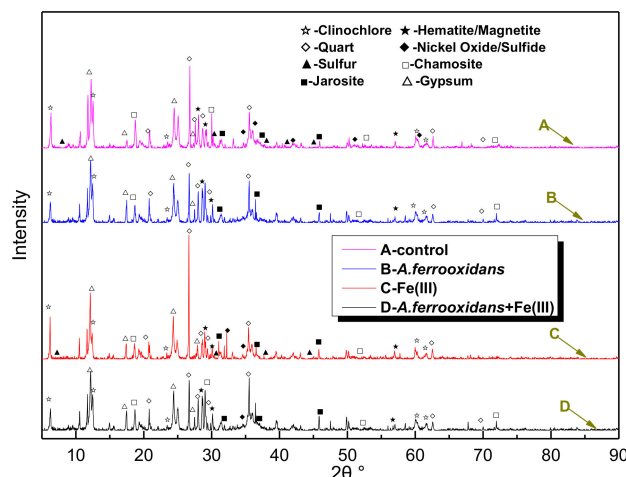


Figure 3. XRD pattern of residues in different leaching system (30 °C; pH 1.8; mole ratio $n_{(S)}/n_{(Mn)}$ 0.67:1; pulp mass fraction 8%; agitation rate 140 r/min; *A. ferrooxidans* amount 1.0×10^8 /mL; Fe(III) concentration 1.0 g/L; processing time 96 h).

3.1.2. Effect of Dominant Factors on Extraction of Ni and Mn

The effects of the molar ratio of $n_{(S)}/n_{(Mn)}$, initial bacteria amount, initial Fe(III) concentration, and pH on the polymetallic nodule and low-grade sulfur-nickel ore-coupled leaching in *A. ferrooxidans* and without *A. ferrooxidans* solution as the extraction efficiency and rate of Ni and Mn, are summarized in Figure 4. Almost 9.7% and 4.9% increases in the leaching efficiency of Ni and Mn for 168 h leaching, respectively, were observed in the presence of *A. ferrooxidans* compared to its absence, suggesting that *A. ferrooxidans* releases more electrons from the sulfur-containing minerals. Figure 4a shows that Mn leaching rate increases with the increase in sulfide ore dosage, but nevertheless, the Ni leaching rate increases and then decreases. This indicates that the insufficiency of the manganese oxide electron acceptor, as well as sulfide ore donor, weakens the corrosion force of the primary battery. A higher initial Fe(III) concentration, comparable to the *A. ferrooxidans* number density, correlates with a faster leaching rate for Ni and Mn. According to the results in Figure 4b,c, 1.0 g/L Fe(III) and 1.0×10^8 /mL *A. ferrooxidans* should be appropriate for the increase in Ni and Mn extraction efficiency. High or low pH is unfavorable to the extraction of nickel and manganese [21]. Hydrogen ion participates in manganese reduction and sulfur oxidation [22]. The high concentration of hydrogen ion leads to a driving force enhancement of the coupled reaction of polymetallic nodule cathode and low-grade nickel sulfide anode. While in the medium containing *A. ferrooxidans*, the extraction rate is faster at pH 1.8 than pH 1.6 due to the relatively higher activity of *A. ferrooxidans*. *A. ferrooxidans* performs manganese extraction weakly compared to nickel at pH 1.6, suggesting a harmful biological activity [23]. However, at pH above 2.0, bio-leaching produces jarosite, covering the mineral surface and resulting in a decrease in the extraction efficiency of valuable metals [24].

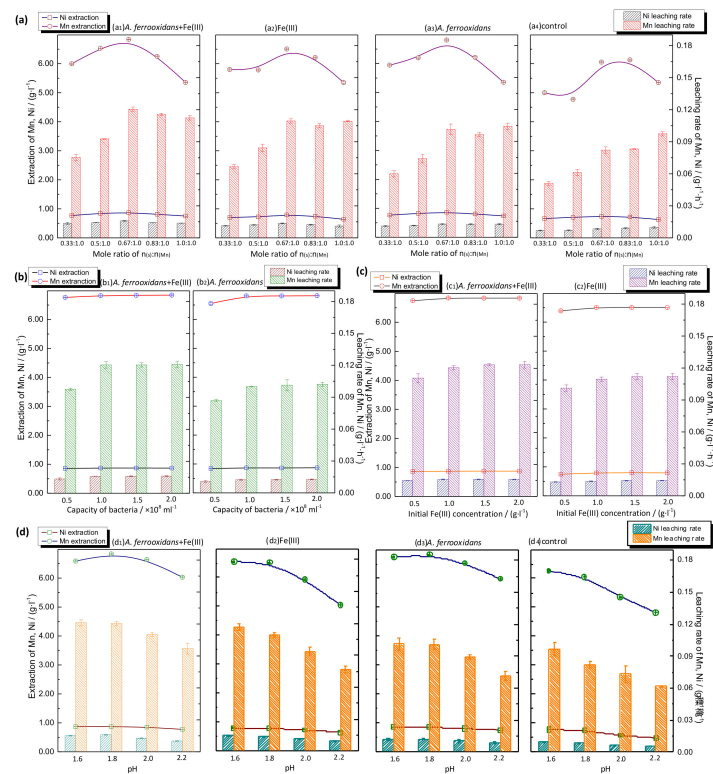


Figure 4. Effects of the (a) molar ratio of $n_{(S)}/n_{(Mn)}$, (b) inoculation amount of bacteria, (c) initial Fe(III) concentration, and (d) pH on Ni and Mn leaching (30 °C; pulp mass fraction 8%; agitation rate 140 r/min; processing time 168 h. (a) *A. ferrooxidans* amount 1.0×10^8 /mL; Fe(III) concentration 1 g/L, pH 1.8; (b) mole ratio of $n_{(S)}/n_{(Mn)}$ 0.67:1, Fe(III) concentration 1 g/L, pH 1.8; (c) mole ratio of $n_{(S)}/n_{(Mn)}$ 0.67:1, *A. ferrooxidans* amount 1.0×10^8 /mL, pH 1.8; (d) molar ratio of $n_{(S)}:n_{(Mn)}$, *A. ferrooxidans* amount 1.0×10^8 /mL; Fe(III) concentration 1 g/L).

3.1.3. Effect of *A. Ferrooxidans* on Ni, Cu, Co, Mn Coupled Leaching

Figure 5 presents the leaching efficiency and rate of the main metals in the presence and absence of *A. ferrooxidans*. Following the quasi-balanced Ni, Cu, Co, and Mn leaching efficiency, it shows an increase of 9.7, 9.8, 4.7 and 4.9%, respectively, in the *A. ferrooxidans* solution compared to the Fe(III) solution without *A. ferrooxidans*, and 16.7, 14.3, 12.5 and 10.4% higher than the control (9 K basic solution) (Figure 5a). Under the most suitable condition (temperature 30 °C; pH 1.8; $n_{(S)}/n_{(Mn)}$ molar ratio of 0.67/1; 8% pulp mass fraction; 140 r/min agitation rate; 1.0×10^8 /mL initial amount of bacteria; 1.0g/L Fe(III) concentration) of the nodule and sulfide simultaneous leaching in *A. ferrooxidans* sulfuric acid solutions for 168 h, the leach liquors contained approximately Ni 0.871 g/L, Cu 0.697 g/L, Mn 6.835 g/L, and Co 0.0688 g/L, corresponding to the leaching ratios of 95.4% Ni, 92.2% Cu, 97.8% Co, and 97.2% Mn. Compared to the control, the average leaching rate of the main elements increased in the presence of Fe(III) and *A. ferrooxidans* for the original 48 h operation. In the *A. ferrooxidans* medium, the leaching rate of Ni, Cu, Co, and Mn increased by factors of 0.48, 0.13, 0.20 and 0.23, respectively, with reference to the control; in the *A. ferrooxidans* + Fe(III) medium, the rates increased by factors of 0.17, 0.50, 0.11, and 0.11 compared to the Fe(III). *A. ferrooxidans* facilitated the coupling corrosion between oxidized and reduction ores.

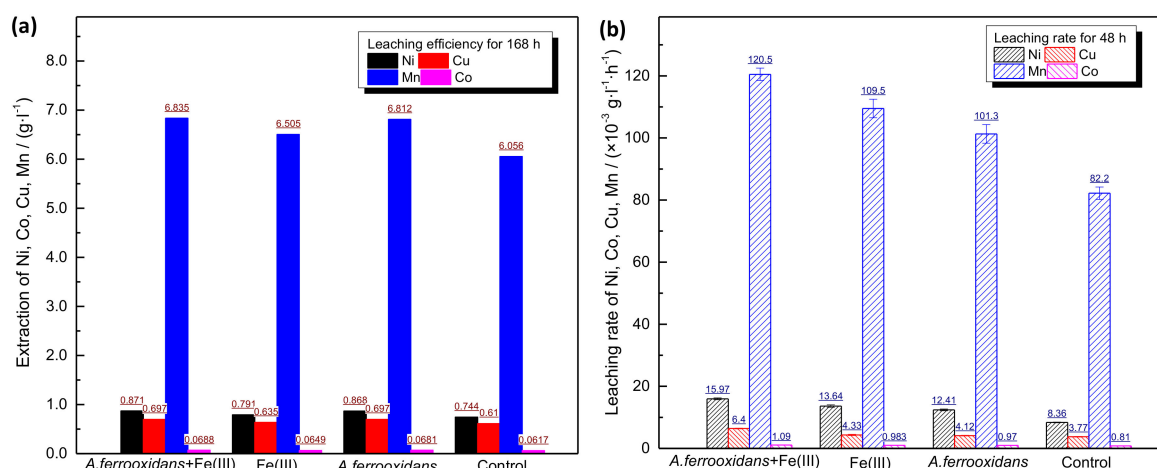


Figure 5. Effect of *A. ferrooxidans* on the (a) extraction efficiencies and (b) average leaching rates of main series metals elements (30 °C; pH 1.8; mole ratio $n_{(S)}/n_{(Mn)}$ 0.67:1; pulp mass fraction 8%; agitation rate 140 r/min; *A. ferrooxidans* amount 1.0×10^8 /mL; Fe(III) concentration 1 g/L).

3.2. Electrochemical Aspects of Low-Grade Sulfur-Nickel Ore and Polymetallic Nodule Corrosion Cell in *A. Ferrooxidans* Bio-Leaching System

3.2.1. Electrode Potential

The open circuit potential-time plots of electrodes in various solutions are illustrated in Figure 6. Both *A. ferrooxidans* and Fe(III) increase the open circuit potential of nickel sulfide. There was a decline in electrode potential when *A. ferrooxidans* was introduced in the Fe(III) solution relative to Fe(III) alone, indicating that *A. ferrooxidans* promotes sulfide oxidation, minimizing the surface concentration of Fe(III). With or without Fe(III), *A. ferrooxidans* increases the potential of the polymetallic nodule. The potential difference between the nodule anode and sulfide cathode shows a rising tendency in the *A. ferrooxidans* and Fe(III)-containing medium.

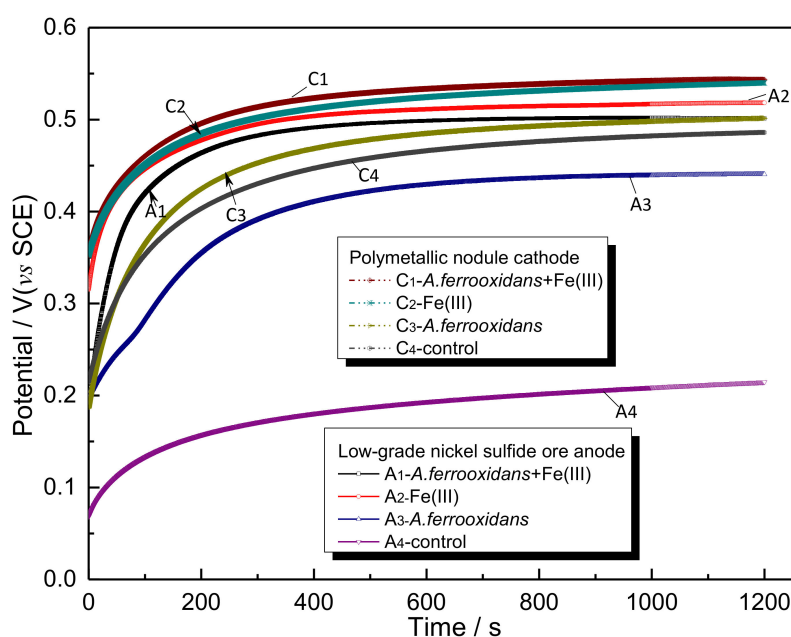
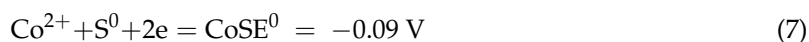
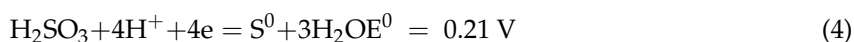
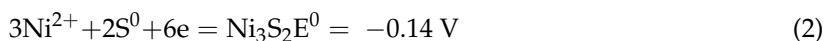
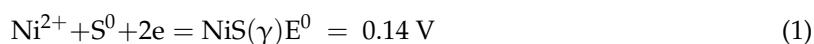


Figure 6. Open circuit potential-time plots of low-grade nickel sulfide ore anode and polymetallic nodules cathode in various solutions (30 °C; pH 1.8; *A. ferrooxidans* amount 1.0×10^8 /mL; Fe(III) concentration 1.0 g/L; scan rate 1 mV/s; counting rate 10/s).

3.2.2. Chemical Reaction

Figure 7 presents cyclic voltammograms of nodule and nickel ore in various solutions. The redox of $\text{Fe}^{3+}/\text{Fe}^{2+}$ of sulfide ore is observed between 0.3 and 0.5 V and the redox of S^0/S^{2-} is between -0.1 and 0.2 V in the Fe(III)-free solution (Figure 7a). Due to the E_{pa} negative shift and E_{pc} positive shift, the potential differences between $\text{Fe}^{3+}/\text{Fe}^{2+}$ and S^0/S^{2-} electrode couples decreased by 33 mV and 32 mV, respectively, in the presence of *A. ferrooxidans*, suggesting that *A. ferrooxidans* promotes the redox dissolution of iron minerals and sulfur minerals. The oxidation peak current of $\text{Fe}^{3+}/\text{Fe}^{2+}$ increases and adsorption peak of S^0 (-0.01 V) decreases, resulting from *A. ferrooxidans* acceleration of the Fe^{2+} and S^0 oxidation.

A significant acceleration of the corrosion and electron-transfer rate of sulfide ore obtained from employing Fe(III) (Figure 7b) is attributed to the reduction potential of $\text{Fe}^{3+}/\text{Fe}^{2+}$, which is higher than the oxidation potential of Me(Ni, Co, Cu, Mn). Compared to the Fe(III) solution, the cathodic peak of sulfide ore appeared at 0.21 V negatively moved 0.15 V and the anodic peak positive shift 0.22 V was observed at 0.74 V in the *A. ferrooxidans* + Fe(III) medium. In the range from 0.1 to 0.6 V the ratio of the cathodic and anodic current peak obtained from the *A. ferrooxidans* + Fe(III) medium was closer to 1, than that from the Fe(III) solution. Those results suggest that *A. ferrooxidans* evokes the polarization of $\text{Fe}^{3+}/\text{Fe}^{2+}$ and accelerates the exchanges rate of $\text{Fe}^{3+}/\text{Fe}^{2+}$ to promote the decomposition of sulfide. The cathodic peak and anodic peak of S^0/S^{2-} appears at approximately -0.03 V and 0.26 V, respectively, in the *A. ferrooxidans*+Fe(III) solution with the Fe(III) medium. There is an adsorption peak of S^0 at 0.01 V and a dissolution peak of S^0 at -0.03 V, suggesting that *A. ferrooxidans* enhances the activity of electron liberation from the elemental sulfur. Furthermore, the anodic peak at -0.26 V could be the equilibrium potential of $\text{S}_2\text{O}_6^{2-}/\text{SO}_4^{2-}$. The probable corrosion of low-grade nickel sulfide anode could be listed as (V vs. SCE) [25,26]:



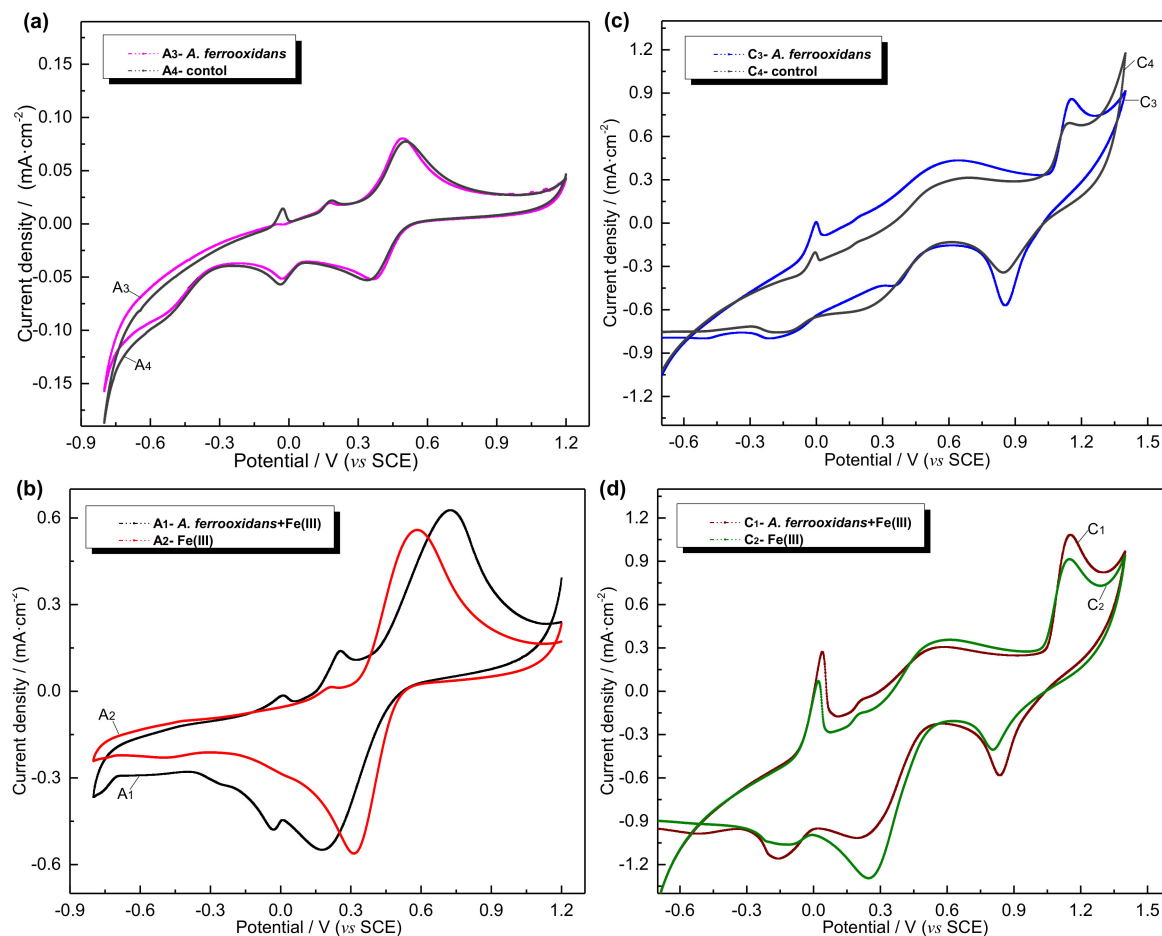


Figure 7. Cyclic voltammetry curves of (a,b) low-grade nickel sulfide ore, (c,d) polymetallic nodules in various solutions (30 °C; pH 1.8; *A. ferrooxidans* amount 1.0×10^8 /mL; Fe(III) concentration 1.0 g/L; scan rate 50 mV/s).

Figure 7c,d indicate that in the presence of *A. ferrooxidans*, the oxidation potentials of the $\text{MnO}_2/\text{Mn}^{2+}$ couple and $\text{Mn(OH)}_3/\text{Mn(OH)}_2$ couple of the polymetallic nodule electrode positively shift by 0.1 V and the cathodic peak current increased more than 90% compared to the absence of *A. ferrooxidans*, suggesting that *A. ferrooxidans* promotes electron transfer or proton diffusion and causes a positive shift of in the Mn(IV) reduction potential. *A. ferrooxidans* motivates a superior iron cycle of the Mn(IV) ore cathode by narrowing the potential difference and balancing the current of $\text{Fe}^{3+}/\text{Fe}^{2+}$, as shown in Figure 7c. In the iron-containing medium (Figure 7d), the $\text{Fe}^{3+}/\text{Fe}^{2+}$ couple performed a negative shift in equilibrium potential and a decrease in the oxidation rate in the presence of *A. ferrooxidans*, because the Mn(IV) acceptor is more active than the Fe(III) acceptor. The anode reactions of the polymetallic nodule could be expressed as(V vs. SCE) [27,28]:

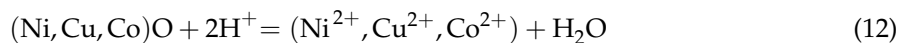
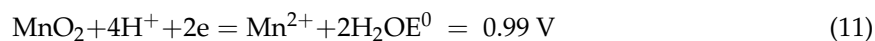
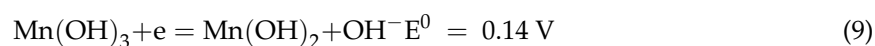


Figure 8 shows the relationship between scan rate (v) and peak current (i_p) in the different systems, with the $i_{pc, Mn}$ nearly at 0.83 V as the cathodic current response of the polymetallic nodule electrode, and the $i_{pa, S/Fe}$ in the range from 0.1 to 0.75 V as the anodic current response of low-grade nickel sulfide electrode. The MnO_2 dissolution rate of the polymetallic nodule electrode increases with scanning rate. The anodic potential of $E_{pc, Mn}$ tends toward positive polarization, $i_{pc, Mn}/i_{pa, Mn} > 1$ and increases. The $i_{pc, Mn}/v^{1/2}$ increases at first, and then decreases, indicating that the MnO_2 dissolution is a controlled prozone reaction, that is to say, $MnO_2 + H^+ + e \rightleftharpoons MnO \cdot OH \rightarrow Mn^{2+}$ [29]. The $i_{pa, S/Fe}/v^{1/2}$ of the low-grade sulfide nickel electrode gradually decreased with scanning rate until it was independent of scanning rate, showing dynamic kinetic current and indicating that the dissolution of minerals is mainly affected by the potential difference.

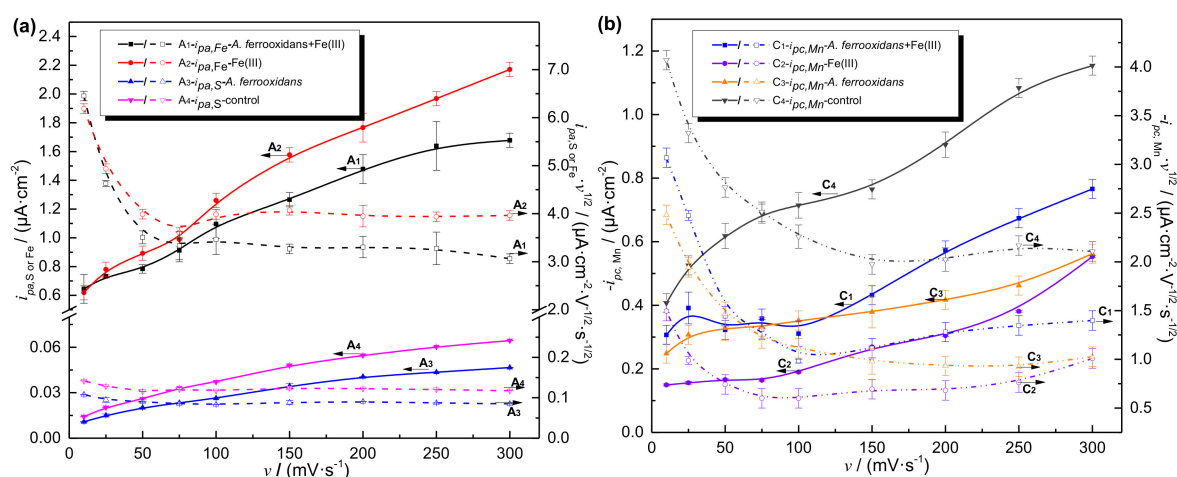


Figure 8. Effect of scan rate i_p vs. v , $i_p \cdot v^{-1/2}$ vs. v , (a) low-grade nickel sulfide ore, (b) polymetallic nodules in various solutions (30 °C; pH 1.8; *A. ferrooxidans* amount 1.0×10^8 mL; Fe(III) concentration 1.0 g/L).

3.2.3. Corrosion Kinetic

(1) Reaction impedance responding to *A. ferrooxidans*

Figure 9 presents impedance spectra for the electrodes in the different solutions. The results for R_{sol} (solution resistance), R_{act} (charge-transfer resistance), and η_{CPE} (dispersion coefficient) are summarized in Table 2. The dissolution of valuable metals from both ores is controlled by interfacial chemical reactions, and the corrosion of both ores markedly increased with the presence of *A. ferrooxidans* and Fe(III). *A. ferrooxidans* and Fe(III) facilitates an approximately 13.2% and 23.3% decrease in the charge-transfer resistance of nickel ore compared to the control, respectively. Combining the previous statements, the decrease in the charge-transfer resistance of sulfide ore possibly results from the *A. ferrooxidans* acceleration of electron transfer, but Fe(III) is derived from the higher potential. The dispersion coefficient corresponding to diffusion efficiency increased with the employment of *A. ferrooxidans* to the solution, suggesting that *A. ferrooxidans* promotes the product layer diffusion. The superior effect of *A. ferrooxidans* on nodule corrosion is due to the higher reaction activity, lower electrochemical impedance, and faster electron transfer. In addition, solution conductivity increases with the use of *A. ferrooxidans*.

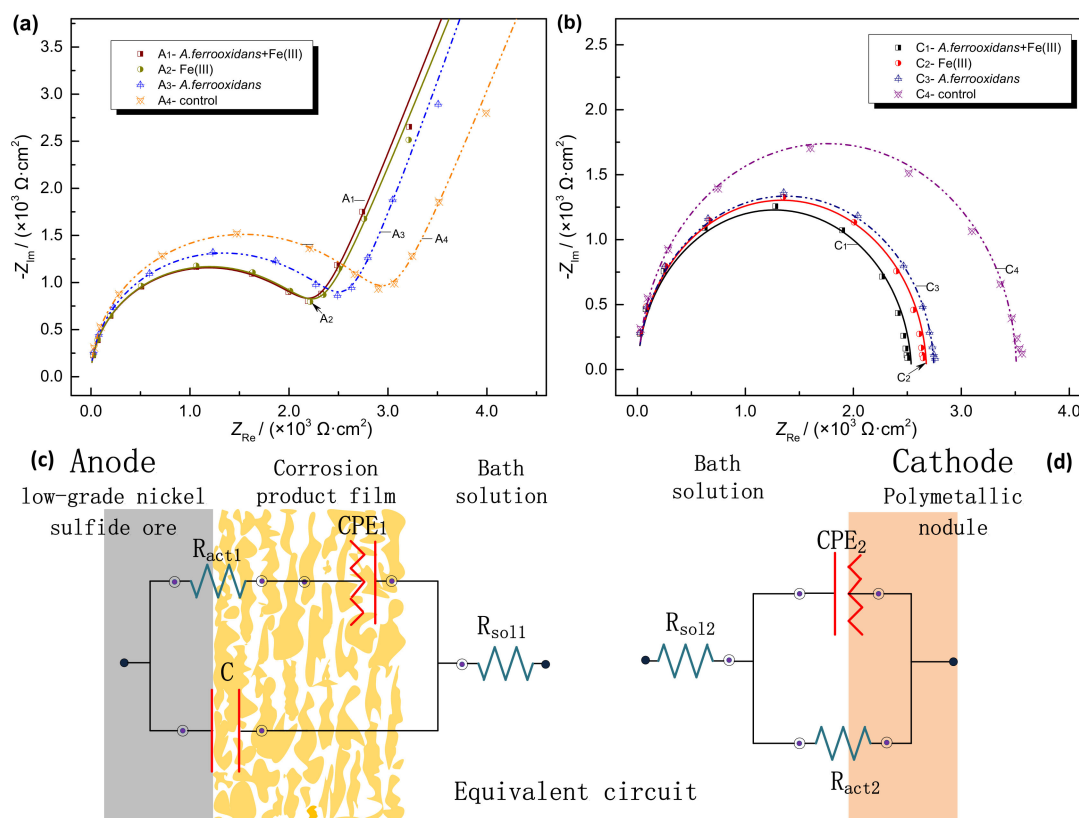


Figure 9. Nyquist plots of (a) and (c) low-grade sulfur-nickel ore and (b) and (d) polymetallic nodule in various solutions (30 °C; pH 1.8; *A. ferrooxidans* amount 1.0×10^8 mL; Fe(III) concentration 1.0 g/L; frequency $0.01 \sim 10^5$ Hz, amplitude 5 mV, bias voltage OPC).

Table 2. Electrode resistance ($\Omega \cdot \text{cm}^2$) obtained from EIS diagrams present Figure 9.

Solution	R_{sol1}	R_{act1}	η_{CPE}	R_{sol1}	R_{act2}
Low-grade nickel sulfide ore			Polymetallic nodule		
<i>A. ferrooxidans</i> + Fe(III)	22.4	2268	0.814	24.6	2532
Fe(III)	21.8	2291	0.799	25.2	2671
<i>A. ferrooxidans</i>	24.3	2586	0.825	26.5	2763
control	27.4	2978	0.795	30.1	3512

(2) Electron charge responding to *A. ferrooxidans*

Figure 10 shows the chronoamperometric plots of ore electrodes in various solutions. The time to achieve a balance of electrode decreased in the presence of *A. ferrooxidans*, and electrodes balanced faster in the *A. ferrooxidans* + Fe(III) solution than in the Fe(III) solution, indicating that *A. ferrooxidans* accelerated electrons transfer. *A. ferrooxidans* or Fe(III) can increase current, driving to a similar equilibrium of polymetallic nodules, illustrating that *A. ferrooxidans* and Fe(III) promote the precursor reaction of MnO_2 electrode dissolution. The steady current of the low-grade nickel sulfide ore electrode increased with admixing *A. ferrooxidans*, and is much higher with the employment of Fe(III) than without Fe(III), showing that Fe(III) increases the electric potential of the system. However, *A. ferrooxidans* accelerates electrons transfer [30,31].

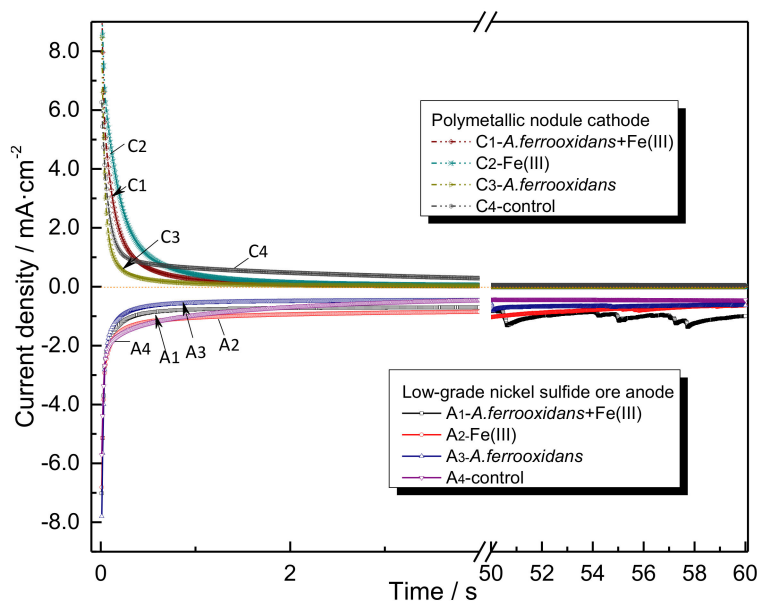
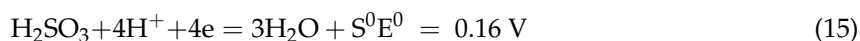
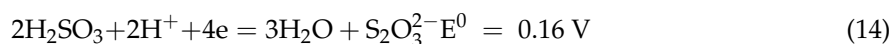


Figure 10. Chronoamperometric plots of low-grade nickel sulfide ore anode and polymetallic nodules cathode in various solutions (30 °C; pH 1.8; *A. ferrooxidans* amount 1.0×10^8 /mL; Fe(III) concentration 1.0 g/L; polarization potential 1.0 V; counting rate 100/s).

(3) Polarization curves responding to *A. ferrooxidans*

The polarization behavior of the electrodes in various solutions is shown in Figure 11. In the presence of Fe(III) (Figure 11a), the corrosion current of nickel sulfide electrode is greater than $5.5 \times 10^{-6} \text{ A} \cdot \text{cm}^{-2}$, far higher than that of the ironless condition. Concretely, the corrosion potential of the electrode in *A. ferrooxidans*+Fe(III) solution is 0.012 V higher than that in the Fe(III) solution, and the reduction current and the exchange current increases, indicating that *A. ferrooxidans* promotes the reduction of $\text{S}_x^{2-}/\text{Fe}^{2+}$. In the iron-free electrolyte, the three Tafel polarization zones in the ranges of -0.8 to 0 , 0 to 0.2 , and 0.2 to 0.6 V correspond to Equations (13)–(15), respectively (vs SCE). The potential, as in Equations (13) and (15), moves in the positive direction, and the corrosion current decreases due to the presence of *A. ferrooxidans*, but in Equation (14) the opposite is true, suggesting that *A. ferrooxidans* can release electrons from low valence S minerals more quickly, causing the generation rate of S^0 to slow or the oxidation of S^0 to high valence soluble ions to accelerate [32].



There was a decrease of almost 0.12 V in corrosion potential and an increase in oxidation current of the polymetallic nodule with the employment of *A. ferrooxidans* in iron-free solutions (Figure 11b). In Fe(III)-containing solution, *A. ferrooxidans* evoked a positive shift of 0.2 V in corrosion potential and displayed a stronger cathodic limiting current of the nodule electrode, which is much higher than in iron-free solutions. The cathodic current is $i_{pc,A.f+Fe} \gg i_{pc,Fe}$ in the potential range from 0.45 to 0.6 V, suggesting that *A. ferrooxidans* improves the ability to be dissolved of Mn(IV) oxide and strengthen the electrochemical driving force of Mn(IV) oxidation sulfide ore.

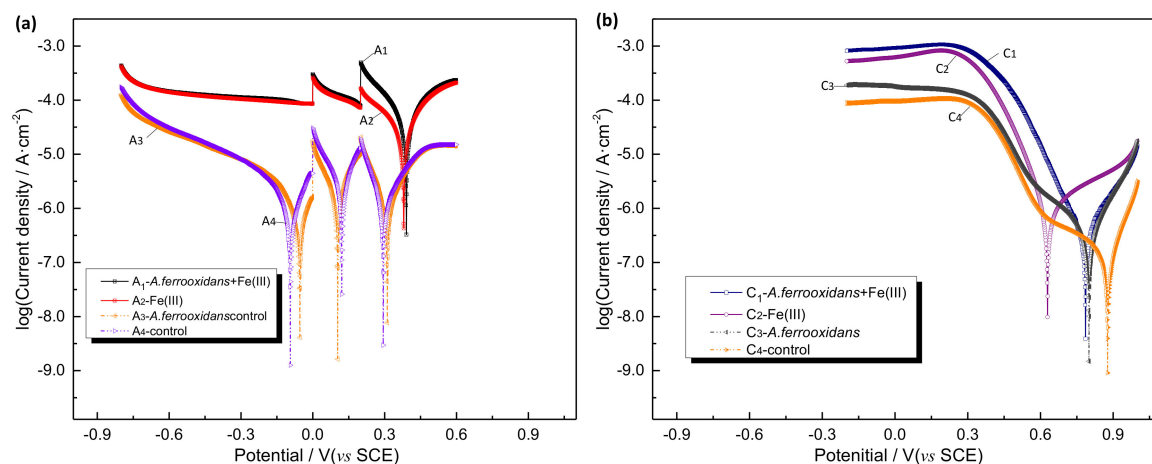


Figure 11. Polarization curves of (a) low-grade nickel sulfide ore and (b) polymetallic nodules in various solutions (30 °C; pH 1.8; *A. ferrooxidans* amount 1.0×10^8 /mL; Fe(III)concentration 1.0 g/L; scan rate 5 mV/s).

3.2.4. Effects of pH and Fe(III) on Coupled Bio-Leaching

Figure 12 shows the effects of pH and Fe(III) on electrode polarization. *A. ferrooxidans* motivates a positive shift in the corrosion potential of the nickel sulfide ore electrode in the iron-free solution, located from -0.8 to 0 V and 0.2 to 0.6 V, indicating that *A. ferrooxidans* promotes the transformation of sulfide to S^0 and S^0 to SO_3^{2-} . The relative over-potential and current increased with pH, appearing at approximately -0.1 V and 0.3 V. Because *A. ferrooxidans* accelerates the change in S^{2-} to SO_4^{2-} , generating H^+ , the reduction of H^+ concentration is beneficial to the conversion of S^{2-} to S^0 and S^0 to SO_3^{2-} . With increased pH, comparing the *A. ferrooxidans* solution with the control, the corrosion potential of the sulfide electrode in the range of 0 to 0.2 V shifts negatively at first, and then positively, but the relative potential difference decreases, suggesting that *A. ferrooxidans* slows the conversion of $S_2O_3^{2-}$ to SO_3^{2-} .

Fe(III) accelerates the pace of sulfur-nickel ore corrosion. Higher pH is correlated with the lower corrosion current, indicating that H^+ concentration affects Fe^{3+} diffusion. Comparing the presence and absence of *A. ferrooxidans* in the Fe(III)-containing solution, i_{pa}/i_{pc} of the former is close to 1, with an optimum value of 0.9886 at pH1.8, indicating that *A. ferrooxidans* facilitates the redox of Fe^{3+}/Fe^{2+} couple. Moreover, the exchange current density of the former is $6.51 \times 10^{-5} A \cdot cm^{-2}$, increased by a factor of 0.45 compared to the latter, showing that *A. ferrooxidans* positively affects oxidation erosion of the nickel sulfide ore electrode.

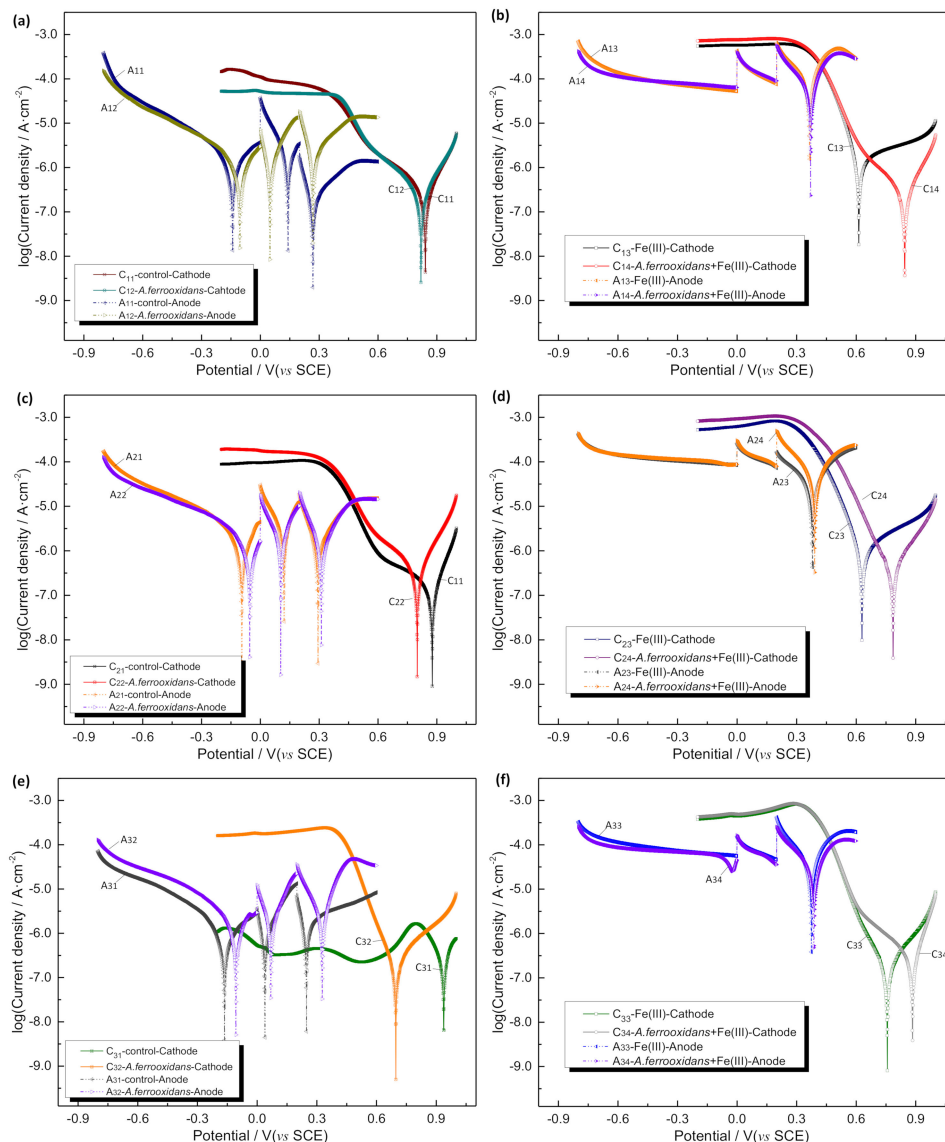


Figure 12. Effect of pH and Fe(III) on dissolution of low-grade nickel sulfide ore anode and polymetallic nodules cathode ((a) pH 1.5, Fe(III)-free; (b) pH 1.5, Fe(III)-containing; (c) pH 1.8, Fe(III)-free; (d) pH 1.8, Fe(III)-containing; (e) pH 2.2, Fe(III)-free; (f) pH 2.2, Fe(III)-containing; 30 °C; *A. ferrooxidans* amount 1.0×10^8 /mL; Fe(III) concentration 1.0 g/L; scan rate 5 mV/s).

In the *A. ferrooxidans* electrolyte, the corrosion potential of polymetallic nodule shifts negatively relative to the control at various pH values, and negative polarization increases with pH, illustrating that *A. ferrooxidans* promotes the migration of protons and electrons. The corrosion potential of the electrode moved positively with the increase in pH in the control solution, suggesting that the decrease in H^+ concentration is unfavorable to the dissolution of Mn(IV) ore. Nevertheless, the potential moves in the negative direction when pH increased in the *A. ferrooxidans* solution. There is a 0.12 V negative shift of corrosion potential and a 45% reduction of corrosion current if the pH increases from 1.8 to 2.2. In addition, at pH 2.2 the corrosion potential in *A. ferrooxidans* is lower than the control by 0.25 V. It is claimed, based on these results, that *A. ferrooxidans* facilitates the diffusion of H^+ to the MnO_2 electrode interface.

Comparing the Fe(III) solution to the control reveals a negative polarization of corrosion potential, illustrating that Fe(III) lowers the level of the reduced difficulty of polymetallic nodules. Corrosion potential positively shifts by employing *A. ferrooxidans* in the Fe(III) solution. Fe(III) facilitates the

dissolution of polymetallic nodule and *A. ferrooxidans* enhances the oxidative activity of polymetallic nodules to increase the potential difference between Mn(IV)cathode and S(-II)anode. Moreover, the corrosion current is maximum valued $3.772 \times 10^{-7} \text{ A}\cdot\text{cm}^{-2}$ at pH 1.8 in the *A. ferrooxidans* + Fe(III) medium.

3.3. Mechanism of Multi-Metal Coupled Bioleaching

Figure 13 presents an E_h -pH diagram for the Me(Mn,Ni,Cu,Co,Fe)-S-H₂O system [33]. Ni, Cu, and Co will dissolve while Mn(IV) reduction may not occur in Region A. If the E_h -pH condition falls into Region B, Mn, Ni, Cu, and Co can be selectively leached from iron, and the activity of *A. ferrooxidans* would be favored. Regions C₁ and C₂ are the thermodynamically superior regions of Mn²⁺, Ni²⁺, Cu²⁺, and Co²⁺, and Fe²⁺ could be ascendant in the latter while Fe³⁺ in the former. By adjusting pH and E_h , so that conditions situate in Region ① of Region C₂ in the figure, it should be possible to disintegrate the surrounded structure of high valence manganese mineral by S_x(-II) or Fe(II), and simultaneously to oxidize sulfide by Mn(IV) or Fe(III). Figure 14 shows the potential and Fe²⁺ concentration of leaching solutions versus time. It is clarified that the selective leaching of Ni, Co, Cu, and Mn from Fe could be interpreted on the basis of the solution potential in Region ① of Region C₂ (Figure 13).

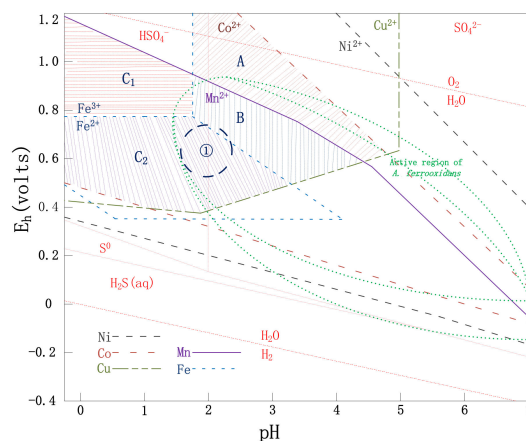


Figure 13. E_h -pH diagrams for Me(Mn, Ni, Cu, Co, Fe)-S-H₂O systems at 25 °C, unit activity 0.01.

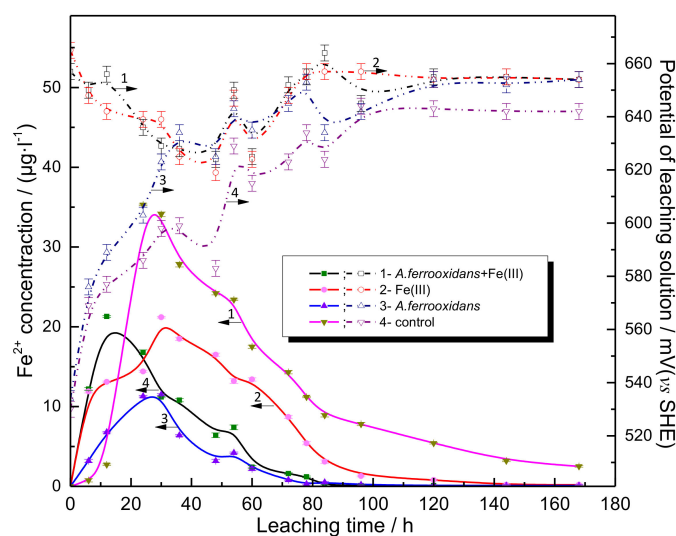
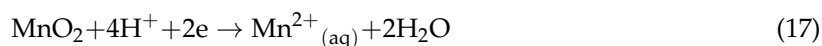
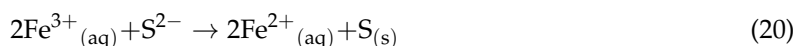


Figure 14. Potential and Fe²⁺ concentration of the leaching solution (30 °C; pH 1.8; mole ratio of $n_{(S)}/n_{(Mn)}$ 0.67/1; initial pulp mass fraction 8 %; agitation rate 140 r/min; initial *A. ferrooxidans* amount 1.0×10^8 /mL; concentration of Fe(III) 1 g/L).

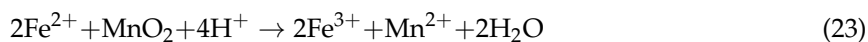
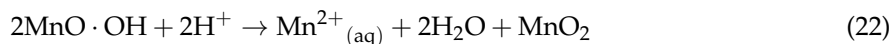
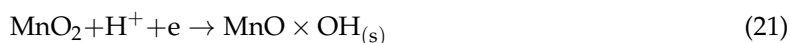
The coupled bioleaching of polymetallic nodule and low-grade sulfur-nickel ore is a process of electrochemical corrosion [34,35]. Figure 15 shows the mutual action model of Me (Ni, Cu, Co, Fe)S anodes and MnO₂ cathodes in *A. ferrooxidans* bio-leaching solutions. In the control vitriolic acid medium without *A. ferrooxidans* and Fe(III), nickel sulfide and manganese oxide constitute the electrodes of the solid primary cell, in which minerals corrode and dissolve by contact and collision. This reaction could be expressed by two half-cell reactions Equations (16) and (17).



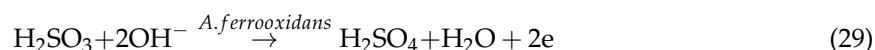
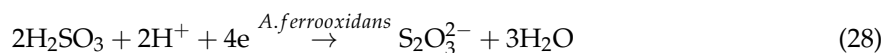
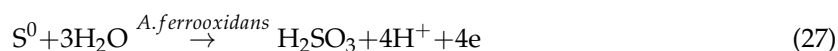
The introduction of Fe(III) will accelerate corrosion due to the redox potential of Fe³⁺/Fe²⁺, which is greater than that of sulfide ore. The anode corrosion reactions of Me(Ni, Cu, Co, Mn, Fe)S can be indicated as Equations (18)–(20):



Because of the oxidizing ability of MnO₂ much higher than Fe³⁺, it can be assumed that the nodule has priority as the electron acceptor in the solution. Cathode routes include the reactions, expressed as Equations (21)–(23).



With *A. ferrooxidans* employed, the conversion reactions, listed as Equations (24)–(29), will be promoted due to the beneficial effect of bacteria on the redox of Fe³⁺/Fe²⁺, MnO₂/Mn²⁺, and sulfur-containing substances [29,32,36–39].



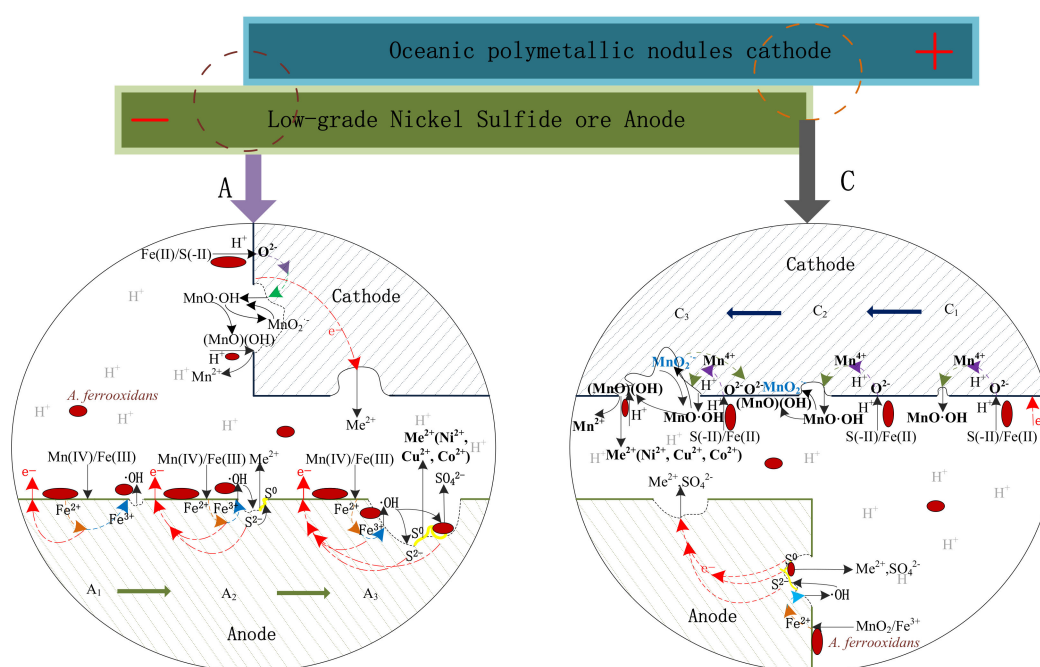


Figure 15. Bio-electrolytic reaction of $\text{MnO}_2/\text{Me}(\text{Ni}, \text{Cu}, \text{Co}, \text{Fe})\text{S}$ in *A. ferrooxidans* bioleaching.

As discussed above, in the *A. ferrooxidans* coupled leaching of the polymetallic nodule and low-grade nickel sulfide ore, potential differences between cathodic and anodic electrodes increases with the presence of *A. ferrooxidans* by the positive shift of the former and negative of the latter. The increase in the coupled leaching efficiencies of the main metals should result from the liberation of more electrons from the sulfide ore by employing *A. ferrooxidans*. *A. ferrooxidans* accelerates the electron transfer and facilitates H^+ diffusion to decrease $Mn(IV)$ dissolution in charge transfer resistance. However, the *A. ferrooxidans* promotion of electron is beneficial for lower impedance of sulfur-nickel ore, and better transformation of the Fe^{3+}/Fe^{2+} couple and S^0/S^{2-} couple, increasing the corrosion of sulfide. Thus, the coupled bio-leaching rates increase in the bacterial solution.

4. Conclusions

The *A. ferrooxidans*-sulfuric acid process has been used to simultaneously extract Ni, Co, Cu, and Mn from polymetallic nodule and low-grade nickel sulfide ore.

A. ferrooxidans increases the potential difference between the polymetallic nodule cathode and low-grade sulfur-nickel ore anode by nearly a 0.10 V positive shift of the former and a 0.12 V negative shift of the latter. In the dissolution of the polymetallic nodule, *A. ferrooxidans* accelerates the diffusion of H^+ and promotes electron transfer, leading to a decrease in impedance and an acceleration of the Mn(IV)-preceding reaction. The corrosion of low-grade nickel sulfide ore shows characteristic kinetic current. *A. ferrooxidans* facilitates the electron exchange by reducing resistance, improving diffusion of product layer, and accelerating transformations of the Fe^{3+}/Fe^{2+} couple and S^0/S^{2-} couple for promoting sulfide mineral corrosion. As a result, the average extraction rates of Ni, Co, Cu, and Mn increased in the biotic solution.

Under the optimal conditions (temperature 30 °C; pH 1.8; molar ratio of $n_{(S)}/n_{(Mn)}$ of 0.67:1; 8% pulp mass fraction; 140 r/min shaking rate; 1.0×10^8 /mL initial amount of bacteria; 1.0 g/L Fe(III) concentration) the leaching efficiencies of Ni, Co, Cu, and Mn were as high as 95.4%, 97.8%, 92.2%, and 97.2%, respectively, representing improvements of 17.1%, 11.5%, 14.3%, and 12.9% compared to the control when the leaching is the quasi-balanced state. The increase in the coupled extraction ratio is due to the greater number of electrons liberated from the sulfide ore by introducing *A. ferrooxidans*.

Author Contributions: Conceptualization, J.-X.K.; Y.-L.F.; and H.-R.L.; Methodology, J.-X.K.; Software, J.-X.K.; Validation, J.-X.K.; Y.-L.F.; H.-R.L.; Z.-W.D.; X.-Y.D. and H.-J.W.; Formal Analysis, J.-X.K.; Investigation, J.-X.K. and Z.-W.D.; Resources, Y.-L.F. and H.-R.L.; Data Curation, J.-X.K. and X.-Y.D.; Writing-Original Draft Preparation, J.-X.K.; Writing-Review and Editing, J.-X.K.; Visualization, J.-X.K. and Y.-L.F.; Supervision, Y.-L.F. and H.-R.L.; Project Administration, Z.-W.D.; Funding Acquisition, Y.-L.F. and H.-R.L.

Funding: The authors thank the sponsoring projects "China Ocean Mineral Resource R and D Association (DY135-B2-15), Major Science and Technology Program for Water Pollution Control and Treatment (2015ZX07205-003), National Natural Science Foundation of China (21176026, 21176242)" for their financial support to publish this paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Meng, L.; Qu, J.; Guo, Q.; Zhang, P.; Han, L. Recovery of Ni, Co, Mn, and Mg from nickel laterite ores using alkaline oxidation and hydrochloric acid leaching. *Sep. Purif. Technol.* **2015**, *143*, 80–87. [\[CrossRef\]](#)
- Wegorzewski, A.V.; Köpcke, M.; Kuhn, T.; Stinikova, M.A.; Wotrub, H. Thermal pre-treatment of polymetallic nodules to create metal (Ni, Cu, Co)-rich individual particles for further processing. *Minerals* **2018**, *8*, 523. [\[CrossRef\]](#)
- Padhan, E.; Sarangi, K.; Subbaiah, T. Recovery of manganese and nickel from polymetallic manganese nodule using commercial extractants. *Int. J. Miner. Process.* **2014**, *126*, 55–61. [\[CrossRef\]](#)
- Senanayake, G. Acid leaching of metals from deep-sea manganese nodules—A critical review of fundamentals and applications. *Miner. Eng.* **2011**, *24*, 1379–1396. [\[CrossRef\]](#)
- Parhi, P.K.; Park, K.H.; Nam, C.W.; Park, J.T.; Barik, S.P. Extraction of rare earth metals from deep sea nodule using H₂SO₄ solution. *Int. J. Miner. Process.* **2013**, *119*, 89–92. [\[CrossRef\]](#)
- Yang, H.Y.; Luo, W.J.; Gao, Y. Effect of *Acidithiobacillus ferrooxidans* on humic-acid passivation layer on pyrite surface. *Minerals* **2018**, *8*, 422. [\[CrossRef\]](#)
- Wei, D.P.; Liu, T.; Zhang, Y.M.; Cai, Z.L.; He, J.T.; Xu, C.B. Vanadium Bioleaching Behavior by *Acidithiobacillus ferrooxidans* from a Vanadium-Bearing Shale. *Minerals* **2018**, *8*, 24. [\[CrossRef\]](#)
- José, A.D.; Jennyfer, S.; Eduardo, L. Bioleaching of arsenic-bearing copper ores. *Minerals* **2018**, *8*, 215.
- Zhang, X.; Feng, Y.L.; Li, H.R. Enhancement of bio-oxidation of refractory arsenopyritic gold ore by adding pyrolusite in bioleaching system. *Trans. Nonferrous Met. Soc. China* **2016**, *26*, 2479–2484. [\[CrossRef\]](#)
- Ghosh, S.; Mohanty, S.; Akcil, A.; Sukla, L.B.; Das, A.P. A greener approach for resource recycling: Manganese bioleaching. *Chemosphere* **2016**, *154*, 628–639. [\[CrossRef\]](#)
- Marrero, J.; Coto, O.; Schippers, A. Anaerobic and aerobic reductive dissolutions of iron-rich nickel laterite overburden by *Acidithiobacillus ferrooxidans*. *Hydrometallurgy* **2017**, *168*, 49–55. [\[CrossRef\]](#)
- Mahmoud, A.; Cézac, P.; Hoadley, A.F.A.; Contamine, F.; Hugues, P.D. A review of sulfide minerals microbially assisted leaching in stirred tank reactors. *Int. Biodeterior. Biodegrad.* **2016**, *119*, 118–146. [\[CrossRef\]](#)
- Clarke, T.; White, G.E.; Edwards, M.J.; Gomez-Perez, L.; Richardson, D.J.; Butt, J.N. Mechanisms of bacterial extracellular electron exchange. *Adv. Microb. Physiol.* **2016**, *68*, 87–138.
- Jiang, T.; Yang, Y.; Huang, Z.; Zhang, B.; Qiu, G. Leaching kinetics of pyrolusite from manganese–silver ores in the presence of hydrogen peroxide. *Hydrometallurgy* **2004**, *72*, 129–138. [\[CrossRef\]](#)
- Kumari, A.; Natarajan, K.A. Electrobioleaching of polymetallic ocean nodules. *Hydrometallurgy* **2001**, *62*, 125–134. [\[CrossRef\]](#)
- Heller, C.; Kuhn, T.; Versteegh, G.J.M.; Wegorzewski, A.V.; Kasten, S. The geochemical behavior of metals during early diagenetic alteration of buried manganese nodules. *Deep-Sea Res. Part I* **2018**, *142*, 16–33. [\[CrossRef\]](#)
- Porter, T.M. Regional tectonics, geology, magma chamber processes and mineralisation of the Jinchuan nickel-copper-PGE deposit, Gansu Province, China: A review. *Geosci. Front.* **2016**, *7*, 431–451. [\[CrossRef\]](#)
- Xie, Q.; Yu, L.; Li, Z.; Liu, H.; Guo, Z.; Wu, Z.; Zhu, H.; Sun, Y.; Khan, A. A novel method of dissolving realgar by immobilized *Acidithiobacillus ferrooxidans*. *Int. J. Miner. Process.* **2015**, *143*, 39–42. [\[CrossRef\]](#)
- Beijing General Research Institute of Mining & Metallurgy. *Analysis of Ore and Nonferrous Metals Handbook*; Metallurgical Industry Press: Beijing, China, 1990. (In Chinese)
- Ding, F.; Zhan, J.; Wang, Z.; Chai, L.; Zhang, C. Simultaneous leaching of low grade bismuthinite and pyrolusite ores in hydrochloric acid medium. *Hydrometallurgy* **2016**, *166*, 279–284. [\[CrossRef\]](#)

21. Chandraprabha, M.N.; Natarajan, K.A. Role of outer membrane exopolymers of *Acidithiobacillus ferrooxidans* in adsorption of cells onto pyrite and chalcopyrite. *Int. J. Miner. Process.* **2013**, *123*, 152–157. [[CrossRef](#)]
22. Madhuchhanda, M.; Devi, N.B.; Rao, K.S.; Rath, P.C.; Paramguru, R.K. Galvanic interaction between sulfide minerals and pyrolusite. *J. Solid State Electrochem.* **2000**, *4*, 189–198. [[CrossRef](#)]
23. José, M.G.; Cantero, D.; Johnson, D.B. Comparison of the effects of temperature and pH on iron oxidation and survival of *Thiobacillus ferrooxidans* (type strain) and a '*Leptospirillum ferrooxidans*'-like isolate. *Process Metall.* **1999**, *9*, 689–696.
24. Leahy, M.J.; Schwarz, M.P. Modelling jarosite precipitation in isothermal chalcopyrite bioleaching columns. *Hydrometallurgy* **2009**, *98*, 181–191. [[CrossRef](#)]
25. Li, H.; Li, C.; Zhang, Z.Q. Decomposition mechanism of pentlandite during electrochemical bio-oxidation process. *Trans. Nonferrous Met. Soc. China* **2012**, *22*, 731–739. [[CrossRef](#)]
26. Ahmadi, A.; Schaffie, M.; Petersen, J.; Schippers, A.; Ranbar, M. Conventional and electrochemical bioleaching of chalcopyrite concentrates by moderately thermophilic bacteria at high pulp density. *Hydrometallurgy* **2011**, *106*, 84–92. [[CrossRef](#)]
27. Wong, A.K.; Chan, R.C.; Nichols, W.S.; Bose, S. Hydrothermal MnO₂: Synthesis, structure, morphology and discharge performance. *J. Electrochem. Soc.* **2005**, *139*, 325–341.
28. Walanda, D.K.; Lawrance, G.A.; Donne, S.W. Kinetics of Mn₂O₃ digestion in H₂SO₄ solutions I NOVA. *J. Solid State Chem.* **2009**, *182*, 1336–1342.
29. Kozawa, A.; Powers, R.A. The Manganese Dioxide Electrode in Alkaline Electrolyte; The Electron-Proton Mechanism for the Discharge Process from MnO₂ to MnO_{1.5}. *J. Electrochem. Soc.* **1966**, *113*, 870–878. [[CrossRef](#)]
30. Rojas, C.; Vargas, I.T.; Bruns, M.A.; Regan, J.M. Electrochemically active microorganisms from an acid mine drainage-affected site promote cathode oxidation in microbial fuel cells. *Bioelectrochemistry* **2017**, *118*, 139. [[CrossRef](#)]
31. Karimi, S.; Ghahreman, A.; Rashchi, F.; Moghaddam, J. The mechanism of electrochemical dissolution of sphalerite in sulfuric acid media. *Electrochim. Acta* **2017**, *253*, 47–58. [[CrossRef](#)]
32. Almeida, C.; Giannetti, B.F. The electrochemical behavior of pyrite-pyrrhotite mixtures. *J. Electroanal. Chem.* **2003**, *553*, 27–34. [[CrossRef](#)]
33. Vu, H.; Jandová, J.; Lisá, K.; Vranka, F. Leaching of manganese deep ocean nodules in FeSO₄–H₂SO₄–H₂O solutions. *Hydrometallurgy* **2005**, *77*, 147–153. [[CrossRef](#)]
34. Ghosh, A.; Gonzalez, E. Theoretical studies on high-valent manganese porphyrins: Toward a deeper understanding of the energetics, electron distributions, and structural features of the reactive intermediates of enzymatic and synthetic manganese-catalyzed oxidative processes. *Isr. J. Chem.* **2010**, *40*, 1–8. [[CrossRef](#)]
35. Kocaman, A.T.; Cemek, M.; Edwards, K.J. Kinetics of pyrite, pyrrhotite and chalcopyrite dissolution by *Acidithiobacillus ferrooxidans*. *Can. J. Microbiol.* **2016**, *62*, 629–642. [[CrossRef](#)] [[PubMed](#)]
36. Quatrini, R.; Appia-Ayme, C.; Denis, Y.; Jedilicki, E.; Holmes, D.S.; Bonnefoy, V. Extending the models for iron and sulfur oxidation in the extreme Acidophile *Acidithiobacillus ferrooxidans*. *BMC Genom.* **2009**, *10*, 394. [[CrossRef](#)] [[PubMed](#)]
37. Chi, A.; Valenzuela, L.; Beard, S.; Mackey, A.J.; Shabanowitz, J.; Hunt, D.F.; Jerez, C.A. Periplasmic Proteins of the extremophile *Acidithiobacillus ferrooxidans* a high throughput proteomics analysis. *Mol. Cell. Proteom.* **2007**, *6*, 2239–2251. [[CrossRef](#)]
38. Sugio, T.; Tsujita, Y.; Hirayama, K.; Inagaki, K.; Tano, T. Mechanism of Tetravalent Manganese Reduction with Elemental Sulfur by *Thiobacillus ferrooxidans*. *Agric. Biol. Chem.* **1988**, *52*, 185–190. [[CrossRef](#)]
39. Elbehti, A.; Brasseur, G.; Lemesleuneur, D. First evidence for existence of an uphill electron transfer through the bc(1) and NADH-Q oxidoreductase complexes of the acidophilic obligate chemolithotrophic ferrous ion-oxidizing bacterium *Thiobacillus ferrooxidans*. *J. Bacteriol.* **2000**, *182*, 3602–3606. [[CrossRef](#)]

