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Transformation of iron in pure culture process of extremely acidophilic microorganisms

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Abstract: Jarosite and extracellular polymer substance generated during pure culture and bioleaching process have been widely accepted the main transformation of decreasing iron in the medium. In the present work, acidophilus bioleaching organisms *Ferroplasma thermophilum, Leptospirillum ferriphilum* and *Acidithioobacillus ferrooxidans* were cultured. It was found that they can live in low pH environment, and more than 10 particles in each cell intracellular nano-particles are synthesized in the cells. By analyzing the morphology and chemical composition of nano-particles, they were found to contain iron, and the three microorganisms belonged to high-yielding strains. The results show that the transformation of the decreasing iron ions is not only generating jarosite, but also taken into cells and synthesizing ferruginous nano-particles.
Key words: jarosite; extracellular polymer substance; bioleaching; ferruginous nano-particles

1 Introduction

Acidithioobacillus ferrooxidans is a kind of acidophilus, autotrophic, obligate aerobic and gram-negative bacterium that thrives optimally at 30 °C and pH 2 [1−4]. *Leptospirillum ferriphilum* is gram-negative bacteria, which can grow in extremely acidic environment and grow optimally in inorganic media within pH range of 1.3−2.0 [5,6]. *Ferroplasma thermophilum* is a kind of acidophilus archaea that thrives optimally at 45 °C and pH 1.0 [7,8]. All those three kinds of microorganisms have the ability of oxidation of ferrous ions and can commonly grow in 9K medium [9].

Jarosite precipitation is a very widespread phenomenon observed in many bacterial cultures, especially in bioleaching process [10]. The jarosites are ammoniojarosites with the formula $MFe_3(SO_4)_2(OH)_6$, where $M=K^+$, Na^+, NH_4^+, Ag^+, or H_3O^+. The formation of jarosite precipitation is a reaction in competition with the hydrolysis reaction giving products of basic ferric hydroxysulphates. In the cultures process of *Ferroplasma thermophilum*, *Leptospirillum ferriphilum* and *Acidithioobacillus ferrooxidan*, the medium is 9K which contains a high concentration of NH_4^+ ions [9], and the jarosites produced are ammoniojarosites with formula $NH_4Fe_3(SO_4)_2(OH)_6$. Abundant iron ions will reduce and form jarosites in the medium.

Extracellular polymer substance (EPS) is also a very widespread phenomenon observed in *Acidithiobacillus* and *Leptospirillum* cultures [11,12]. The extracellular polymeric substances of both species mainly consist of neutral sugars and lipids [13]. The functions of the exopolymers seem to mediate attachment to a (metal) sulfide surface, and to concentrate iron ions by complexation through uronic acids or other residues at the mineral surface, thus allowing an oxidative attack on the sulfide. In addition, it also contains iron ions [13,14].

Small-angle X-ray scattering [15,16] is well designed to study the nanoscale structures in a material, which is especially appropriate to measure nanoparticles because of their nanoscale sizes. In addition, nano-particles are also the focus research object to seek after

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nucleation mechanism and growth manner of materials. LI et al [15] studied the use of the small-angle X-ray scattering technique to probe the nucleation and growth regularity of nanomaterials. WANG et al [17] studied the size and shape evolution of gold nanoparticles in aqueous solution by using real-time small-angle X-ray scattering and ultraviolet-visible spectra at the 1W2A small-angle X-ray scattering station of Beijing synchrotron Radiation Facility, China.

Organisms have evolved complex strategies to maintain optimal intracellular iron pools without compromising cell viability, which include iron-dependent gene regulation using an array of transcriptional regulators from Fur, DtxR and RirA families, iron acquisition in either free form (e.g. Fe21/Mn21 NRAMP family transporters) or sequestered by small molecules (siderophores), intracellular storage proteins such as ferritins, and readily inducible defenses against free-radical formation [15,16].

In the present work, the transformation of decreasing iron in the medium was investigated. The intracellular nano-particles were observed by transmission electron microscope (TEM), the chemical composition of nano-particles was analyzed by energy spectrum analysis, and the grain size of nano-particles was analyzed by small angle X-ray scattering.

2 Materials and methods

A. ferrooxidans f1, *F. thermophilum* $L1^T$ (EF062309) and *L. ferriphilum* YSK used in this work were provided by the Key Laboratory of Biohydrometallurgy, Ministry of Education, China. The strains were cultivated in basal salts of modified 9K medium, the pH of *A. ferrooxidans* f1 was adjusted to 2.0, by adding 50% H_2SO_4 (volume fraction), and the culture temperature was 30 °C; the pH of *F. thermophilum* $L1^T$ was adjusted to 1.0, and the culture temperature was 45 °C; the pH of *L. ferriphilum* YSK was adjusted to 1.6, and the culture temperature was 45 °C. $FeSO_4{\cdot}7H_2O$ was added into the medium, after that these microorganisms were cultured at rotation speed of 170 r/min. Additional 0.2% yeast extract was added to *F. thermophilum* $L1^T$.

Ultrathin sections were obtained with an ultramicrotome (LKB-5, LKB, Sweden) and then stained with uranyl acetate and lead citrate. The morphologies were observed by TEM (FEI Tecnai Spirit) at an accelerating voltage of 80 kV.

A drop of cell was deposited onto a copper grid (62 μm) covered by a carboncoated formvar film. The cells were allowed to settle on the grid for 10 min, and excess liquid was removed with a filter paper. All grids were rinsed at least twice with distilled water before TEM observation. In case of negative staining, wet grids were treated with a drop of negative staining solution (1.5% uranyl acetate) for 1 min and then observed by TEM (JEM2100F) at an accelerating voltage of 200 kV.

The concentrations of total iron in solution were measured by atomic absorption spectrometry, the ferrous iron concentration was determined by titration with potassium dichromate and the ferric iron concentration was equal to the difference between the concentrations of total iron and ferrous iron. The grain size of nano-particles was analyzed by small angle X-ray scattering at the small angle scattering experiment station (1W2A) at Beijing Synchrotron Radiation Facility, China.

3 Results and discussion

3.1 Morphology of nano-particles

TEM observation was used to analyze the morphology inside the microorganisms. As shown in Fig. 1, nano-particles are seen in *A. ferrooxidans f*1, *F. thermophilum* $L1^T$ and *L. ferriphilum* YSK. The arrangements of the nano-particles inside the three strains are different, the arrangement of the nano-particles is random and disperse in the *A. ferrooxidans* f1 and *L. ferriphilum* YSK. However, in *F. thermophilum* $L1^T$, the nano-particles are gathered. The size of the nano-particles is inhomogenous, and there are more than 10 nano-particles in each cell.

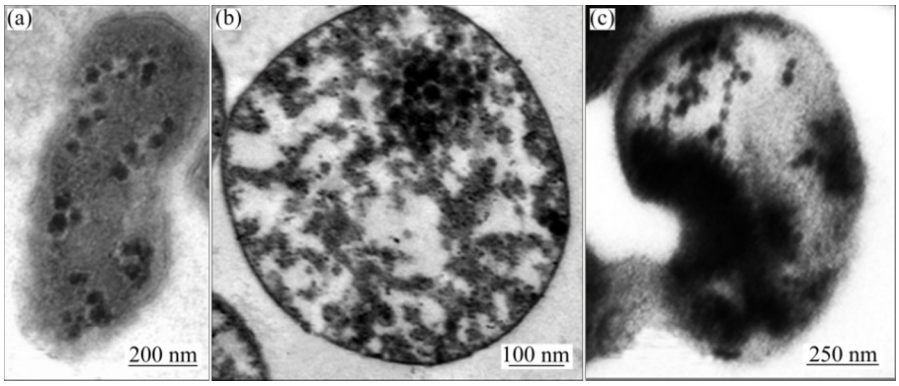

Fig. 1 TEM images of microorganisms: (a) *A. ferrooxidans*; (b) *L. ferriphilum*; (c) *F. thermophilum*