Peculiarities of Adhesion and Bioleaching of Pyrite by New Isolated Leptospirillum Spp. Bacteria

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Abstract

Leptospirillum sp. str. ZC isolated from bioleaching pulp of zinc concentrate is capable of oxidizing Fe (II) with the optimal temperature of 37oC. In pure culture bacteria is unable to oxidize pyrite, however, its growth together with sulfur oxidizing A.albertensis str.SO -2 significantly enhances the efficiency of pyrite bioleaching. The correlation between pyrite biooxidation and adhesion of Leptospirillum spp. bacteria on mineral surface has been found. It has been revealed that in case of mixed culture the cells of Leptospirillum sp. str. ZC are adhered on pyrite surface only after its initial colonization by sulfur oxidizing bacteria. It is supposed that bioleaching of pyrite by Leptospirillum spp. bacteria occurs by means of indirect contact leaching.

Keywords

Leptospirillum spp. bacteria - bacterial adhesion on pyrite- pyrite biooxidation

1. Introduction

Biooxidation is a pretreatment of sulfide minerals during which lattice of pyrite is destroyed by bacteria, gold is released and becomes available for future extraction by cyanidation [7]. Recently, studies have shown that dominating iron oxidizing bacteria in gold bearing arsenopyrite and pyrite biooxidation reactors operating over 40oC are Leptospirillum spp. bacteria (mainly Leptospirillum ferriphilum) [6, 14, 17, 23]. High Fe3+/Fe2+ ratio, elevated temperatures (40°C), as well as extremely low pH values (pH 1.0) are the most favourable for the growth of bacteria of the genus Leptospirillum [10, 18]. At present genus Leptospirillum includes: Leptospirillum ferrooxidans (Group II I group), Leptospirillum ferrirophilum, “Leptospirillum rubarum” (Group II) and “Leptospirillum ferrodiazotrophum” (Group III). Bacteria of the genus Leptospirillum are Gram negative acidophilic motile vibrios which fix carbon dioxide using the energy of oxidation of Fe (II) ion [2, 5, 6, 12].

Recently, scientists emphasize the importance of bacteria of the genus Leptospirillum and their mixed cultures with other bacteria in the biooxidation and bioleaching processes at temperatures above 40°C [2, 9, 16, 21].

Currently, it is considered that bacterial leaching of sulfide minerals is mostly taken place by indirect “contact” mechanism. According to this mechanism the oxidation of minerals is implemented by Fe (III) ions complexed with extracellular polymeric substances:

\[
\text{MS} + 2\text{Fe}^{3+} \rightarrow \text{M}^{2+} + \text{S}^0 + 2\text{Fe}^{2+}
\]

Thus, adhesion and biofilm formation by chemolithotrophic bacteria is essential for efficient biooxidation or bioleaching of sulfide minerals [19, 20, 21]. The extracellular polymeric substances of biofilm mediate cells attachment on mineral, as well as electrochemical reactions taking place on mineral surfaces during bioleaching processes [15, 20]. However, there are no data concerning the adhesion of Leptospirillum spp. bacteria on pyrite, as well as evidence for direct relation between their adhesion rates and intensity of mineral bioleaching processes.

New Leptospirillum spp. bacterium has been isolated from leaching pulp of zinc concentrate which is capable to oxidize Fe (II) ion in the range of temperatures 30-40°C.

The aim of the work was to study the main biological properties of isolated Leptospirillum spp. bacteria and their ability to adhere on sulfide minerals with reference to efficiency of pyrite biooxidation and bioleaching processes.

2. Materials and Methods

The isolation of Leptospirillum spp. bacteria: For the isolation of Leptospirillum spp bacteria 9K media with ferrous iron as a source of energy [1] was infected with samples from bioleaching pulp of zinc concentrate and incubated at 37°C for 7-10 days.

Pure cultures were obtained by transferring the yellow and yellow-brown colonies growing on Manning [1] and FeTSB0 [13] solid media to the above mentioned liquid medium.

Bioleaching of pyrite: For sulfide mineral bioleaching experiments new isolated Leptospirillum sp. strain ZC, as well as previously described sulfur oxidizing
Acidithiobacillus albertensis str. SO-2 were used [3]. The bacterial strains have been grown on 9K medium containing Fe^{2+} or S^{2-} as an energy source. In the logarithmic phase of growth the cells were collected by centrifugation at 6000g for 10 minutes. Biomass collected has been washed with acidified 9K medium and resuspended in the same medium. Pyrite (FeS_{2}) from Shamlugh ore deposit in Armenia ground to 43-63μm was used for leaching experiments. Pyrite grains have been placed into 250 ml flasks, moistened with distilled water and sterilized. Then 50 ml 9K medium without Fe^{2+}, pH 2.0 adjusted by H_{2}SO_{4} and bacterial suspension has been added to the flasks. The pulp density (S:L) was calculated as pyrite mass ratio to the volume of the medium. The bioleaching experiments have been carried out at 37°C in the periodic mode and shaking conditions (180 rev/min.). The intensity of pyrite oxidation has been estimated by the quantity of Fe^{3+}, Fe^{2+} ions being transferred to the medium.

**Bacterial adhesion:** Bacterial adhesion on the surface of pyrite has been studied during 24 hours. The quantity of planctonic cells was determined by direct calculation under the microscope or by the titration in serial tenfold dilution and further examination by Mac-Kredy’s table. The number of adhered cells has been determined as a difference between inserted primary titre of bacterial cells and the cells observed in the liquid phase for some time later. The adhesion rate has been determined as a percentage of adhered cells against their initial number [4].

**Analyses of metal ions:** Fe^{2+} and Fe^{3+} ions have been determined by complexometric titration by Trilon B, total iron ions have been determined by atomic-absorption spectrofotometer AAS 1N using air-propane-butane flame.

### 3. Results and Discussion

**Isolation of Leptospirillum spp. bacteria:** An original strain of Leptospirillum spp. bacteria has been isolated from the pulp of bioleaching of zinc concentrate. The cells are vibrios in the size 0.12-0.13 x 0.6-1.0 μm (Figure 1).

![Microphotography of Leptospirillum sp. strain ZC stained by Cristalline Violet](image)

*Figure 1. Microphotography of Leptospirillum sp. strain ZC stained by Cristalline Violet (1 pixel (px) = 263.6 μm)*

The growth of isolated Leptospirillum sp. strain ZC was observed in the range of temperature 30-40°C with the optimal temperature of 37°C.

The influence of pH on the growth of bacteria and its iron oxidation activity has been studied during the first days of cultivation until no significant change in pH value occurs. Optimal pH value was 2.0, the lower limit for growth was pH 1.4.

**Adhesion of Leptospirillum sp. strain ZC on pyrite and bioleaching of pyrite:** Bioleaching of pyrite has been studied by pure cultures of new isolated iron oxidizing bacteria Leptospirillum sp. strain ZC and its mixed culture with sulfur oxidizing bacteria A.albertensis strain SO-2 isolated from pulp of bioleaching of zinc concentrate.

It has been indicated that pure culture of Leptospirillum sp. strain ZC show very low activity in the oxidation of pyrite even in the presence of 0.5 g/l Fe^{2+} which doesn’t differ from uninoculated control. Any considerable activity of pyrite oxidation has not been detected also with pure culture of A. albertensis strain SO-2. However, in case of together growth of Leptospirillum sp. strain ZC with A.albertensis strain SO-2 the activity of mixed culture exceeded control, pure cultures of Leptospirillum sp. strain ZC and A.albertensis strain SO-2 about 40-50 and 15-20 times, respectively (Figure 2).

The results obtained allow to conclude that Leptospirillum sp. strain ZC shows high activity of pyrite oxidation only when grows in association with sulfur oxidizing bacteria.

![Graph showing bioleaching of pyrite](image)

*Figure 2. Bioleaching of pyrite (FeS_{2}) without bacteria (1), by Leptospirillum sp. strain ZC (2), Leptospirillum sp. strain ZC in the presence of 0.5g/l Fe^{2+} (3), A.albertensis strain SO-2 (4) and their mixed cultures (5)*

Studies carried out have shown that in case of pure and mixed cultures the adhesion of A.albertensis strain SO-2 on pyrite surface achieves maximum rate 99.99% in 30 minutes after inoculation (Table 1,2).

The adhesion of Leptospirillum sp. strain ZC on pyrite surface in case of mixed culture achieved 90,1% only in 24 hours after inoculation while in case of pure culture in the presence of ferrous ion adhesion of Leptospirillum has not entirely been observed (Table 2).
Table 1. Adhesion of pure culture of A.albertensis strain SO-2 on pyrite

| Duration, minutes | Number of planctonic cells of A. albertensis SO-2, cell/ml | Number of adhered cells of A. albertensis, cell/ml | Rate of adhesion, % |
|-------------------|-------------------------------------------------------------|---------------------------------------------------|-------------------|
| 0                 | 7,5 x 10^8                                                  | 0                                                 | 0                 |
| 30                | 5,0 x 10^5                                                  | 7,49 x 10^8                                       | 99,93             |
| 60                | 1,2 x 10^5                                                  | 7,499 x 10^8                                      | 99,98             |

Table 2. Adhesion of mixed culture of Leptospirillum sp. strain ZC and A.albertensis strain SO-2 on pyrite

| Duration | Number of planctonic A. albertensis str. SO-2, cell/ml | Extent of adhesion, % | Number of planctonic cells of Leptospirillum sp.str. ZC, cell/ml | Extent of adhesion, % | Number of planctonic cells of Leptospirillum sp.str. ZC, cell/ml in the presence 0,5g/l Fe2+ | Extent of adhesion, % |
|----------|--------------------------------------------------------|----------------------|---------------------------------------------------------------|----------------------|------------------------------------------------------------------------------------------|----------------------|
| 0        | 7,5 x 10^8                                            | 0                    | 1,32 x 10^6                                                   | 0                    |                                                                                         | 0                    |
| 30 minute| 1,2 x 10^7                                            | 98,4                 | 1,2 x 10^6                                                   | 9,1                  | 1,32 x 10^6                                                                              | 0                    |
| 1 hour   | 5,0 x 10^4                                            | 99,99                | 1,0 x 10^5                                                   | 24,2                 | 3,0 x 10^7                                                                              | 0                    |
| 24 hour  | -                                                      | -                    | 1,2 x 10^5                                                   | 90,1                 |                                                                                         | -                    |

Thus, the results obtained indicate that bioleaching of pyrite is directly connected with the ability of Leptospirillum sp. strain ZC to adhere on mineral surface in addition the efficiency of bioleaching of iron from pyrite is correlated with the amount of adhered cells.

The results indicate also that in case of mixed culture pyrite is previously colonized by sulfur oxidizing bacteria and then the adhesion of Leptospirillum sp. strain ZC on pyrite surface is observed. It is known that sulfur oxidizing bacteria more likely adhere on hydrophobic surfaces. As iron ions have been removed from the surface during initial treatment of pyrite (by boiling hydrochloric acid), it is assumed that some sulfur particles remained on the pyrite surface gave some hydrophobicity to pyrite which explains its rapid colonization by sulfur oxidizing bacteria, as well as the lack of adhesion of Leptospirillum sp. strain ZC. It is known that the adhesion of Leptospirillum sp. strain ZC on solid surfaces mainly occurs by means of electrostatic gravitation [20].

It has been reported that in the absence of sulfur oxidizing bacteria sulfur accumulates on the surface of sulfide minerals and can cause formation of thick layer which has a negative impact on bioleaching kinetics [8, 11]. In this respect the contribution of A.albertensis strain SO-2 in the process of rapid dissolution of pyrite can be explained by the fact that A.albertensis like other sulfur oxidizing bacteria oxidizes sulfur – the intermediate of pyrite leaching process converting it to sulfuric acid thus preventing or reducing the accumulation of sulfur on pyrite surface. At the same time removing hydrophobic layer of sulfur from pyrite surface the favourable conditions for the adhesion of Leptospirillum spp are created. Leptospirillum spp. strain ZC growing in mixed culture with A.albertensis strain SO-2 resulted in promotion of adhesion and initiation of oxidation rate of pyrite and significant enhance of leaching intensity of pyrite.

4. Conclusion

Thus, we can conclude that new isolated Leptospirillum sp. strain ZC shows a high activity of pyrite oxidation only when grows together with sulfur oxidizing A.albertensis strain SO-2 bacteria. The adhesion of Leptospirillum sp. strain ZC on pyrite surface is a necessary prerequisite for its rapid bioleaching. Moreover, it has been revealed that dissolution of pyrite is correlated with the quantity of adhered cells of Leptospirillum spp. bacteria on its surface. Therefore, we can assume that leaching of pyrite by Leptospirillum spp. bacteria perhaps occurs mainly by indirect contact mechanism [19, 20].

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