Bacterial leaching of anodic slime by Thiobacillus ferrooxidans based on bioextraction of selenium-A case study

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ABSTRACT
Bacterial leaching of an anodic slime from Sarcheshmeh Copper Mine of the Southern Iran Contains the three compounds CuSe,Ag_2Se and CuAgSe. The three compounds were studied in shake flask experiments using Thiobacillus ferrooxidans strain isolated from acid nine drainage of the mine sites. The Fe^{2+} grown T.ferrooxidans isolates the dissolved CuSe preferentially over Ag_2Se and CuAgSe leaching 52-58% of Cu and 43-54% of Se from the anodic slime within 25 days. The anodic slime adapted T.ferrooxidans started dissolving Cu and Se without a lag period. The extraction of Se was still much lower in the low-phosphate medium, while in the high-phosphate medium it approached the value obtained with T.ferrooxidans. It was found that T.ferrooxidans dissolve selenium from the anodic slime by the conversion of CuSe to Cu^{2+} and Se^0 . Also the Thibacillus ferrooxidans dissolved selenium to selenite and selenate.

INTRODUCTION
Selenium was formally discovered in 1817 by two Swedish Chemists, Berzelius and Gahn. Selenium belongs to group VIA of the Periodic Table and has been classified as a metalloid, having properties of both a metal and nonmetal [1]. One of the most important sources of selenium is in the anodic slime that's produced of copper electrolysis. Selenium is extracted from the anodic sludge by an alkali leaching, neutral roasting, oxidation roasting, acidic roasting, alkali roasting and chlorination process in usual methods[3].
Thiobacillus ferrooxidans is the major microorganism important in the leaching of minerals[4]. Thiobacillus ferrooxidans in currently used in hydrometallurgy or biohydrometallurgy for the recovery of copper, uranium and another metals from sulfide – bearing material[5].
The S^0 and Fe^{2+} can be oxidized by T.ferrooxidans to SO_4^{-2} and Fe^{3+} , respectively. The acidic pH of leaching media is of critical importance since the bacterium can grow only at pH 1-5 with an optimum at pH 2.3-2.5 [7].
The oxidation of selenium has been studied in copper (II) Selenide by Thiobacillus ferrooxidans. Thiobacillus ferrooxidans can derive its energy from the oxidation of copper selenide. In this metabolic oxidation process, copper goes into solution and elemental selenium is deposited[5].
The other biooxidation studied of selenium reported the oxidation of Se^0 to SeO_3^{2-} and trace amounts of SeO_4^{2-} by a heterotrophic bacterium, Bacillus megaterium[9].
The oxidation of Se^0 has been studied in several soil and sediment experiments [10-14]. We have carried out studies on the bacterial leaching of selenium from a anodic slime from copper electrolysis process in Sarcheshmeh Copper Industry (Located in South of I.R.Iran) using T. ferrooxidans isolate. The results suggest that T.ferrooxidans leach anodic slime and open up a possibility of preferential leaching of *Se* by choice of the organisms and leachning conditions.
In this article the results obtained in shake flask leaching experiments are reported. The column leaching experiments will be reported elsewhere.

MATERIALS AND METHODS
Anodic slime
The anodic sludge used was obtained from the Refinery (electrolysis process site) of Sarcheshmeh Copper Industry Complex. The slime contained *CuSe, CuAgSe* and Ag_2Se that analysis with *XRD*(SEIFERT XRD 3003) system. The elemental analysis was *10%Se, 15%Cu, 6.8%Pb, 5.2%Sb, 3.7%As, 6.6%Si, 50.9%Ba, 0.1Fe* and the rest for *Ag* and *Au*.

Media
The medium used for the growth of T.ferrooxidans was a modified 9k medium (*M9kSe*)(g/Lit): 33.3 $FeSO_4.7H_2O$; 0.1 K_2HPO_4; 0.4 $(NH_4)_2SO_4$; 0.4 $MgSO_4.7H_2O$; [4], 0.1 Na_2SeO_3 and adjusted to pH 2.3 with H_2SO_4.

Two different media, The high – phosphate (HP) medium and the low phosphate (LP) medium, were used for leaching experiments. The HP medium was the M9kSe medium without ferrous sulfate, and the LP medium contained (mg/Lit): 66$(NH_4)_2SO_4$; 123$MgSO_4 \cdot 7H_2O$, and 35 K_2HPO_4 and was adjusted to pH 2.3 with H_2SO_4 [4].

Culture Procedures

Iron – oxidizing bacterial isolates (*Se-1 & Se-2*) were obtained from water and sediment samples from Shoor River in the south of Sarcheshmeh Copper Mine. Each two of These (*Se-1 & Se-2*) were able to grow either an iron and were considered as T. ferrooxidans.

Isolation or growth of T. ferrooxidans was carried out in 500-ml Erlenmeyer flasks containing 150ml *M9kSe* medium with 20-ml portions of inoculum (either water sample or sediments solution or previous culture). The flasks were incubated at 32 ^{o}c on a rotary shaker at 150rpm for several days.

Leaching Experiments

Shake flask leaching experiments were carried out in 500-ml Erlenmeyer flasks containing 150ml medium (HP or LP) plus 1 gram anodic sludge. The anodic sludge flasks were inoculated with 20ml T.ferrooxidans to make final 11% anodic slime slurry. The flasks were incubated at 32 ^{o} c on a rotary shaker at 150rpm.

Sample Analysis

During the course of the leaching experiments, 5-ml samples of slurry were collected from the shake flasks and immediately frozen in order to halt bacterial activity.The samples were thawed and filtered through a Whatman No.1 filter paper to remove anodic slime particles and then centrifuged at 1000g for 15min in order to remove any bacterial cells. The resulting clear samples were then analyzed for *Se* by Atomic Absorption Spectrophotometer Hydride Generation at the *Sharif University of Technology* (Biochemical Engineering & Environmental Control Center- laboratory) and *Cu* by Atomic Absorption Spectrophotometry at the *Azad Unviersity Research and Science Campus* laboratory.

RESULTS AND DISCUSSION

Leaching by Iron – Grown Isolates

Thiobacillus ferrooxidans isolates grown on ferrous iron were not able to leach selenium from anodic sludge in the LP medium. In the initial leaching experiments, the HP medium (o.6mM phosphate) with its better buffering capacity had to be used. Figure 1 illustrates a typical time course leaching experiment with the bacterial isolate *Se-1*. All isolates showed a lag of 7-8 days before commencement of leaching even though the ferric iron initially present in the inoculum must have caused some solubilization. Apparently, a period of adaptation was required in all isolates before growth on the anodic slime. As can be seen from Figure 1 and Table (1), *Se* was extracted with *Cu*. The *Cu-Se* extraction ratios were on the order of 1.2 after 25 days based on the *Cu – Se* anodic sludge content ratio of 15/10 = 1.5. This indicates that *CuSe* was solubilized preferentially over *CuAgSe* and Ag_2Se in anodic slime.

Table I. Leaching by iron-grow isolates. [a]

Bacterial Strain	Metals Extracted (mg/ 1g anodic sludge) Cu			Se			*Cu-Se Extraction* Percentage Ratio		
	10d	*20d*	*25d*	*10d*	*20d*	*25d*	*10d*	*20d*	*25d*
Se-1	22	65	78	15	43	66.5	1.46	1.51	1.17
Se-2	22	65	77	15	41	65.8	1.46	1.58	1.17

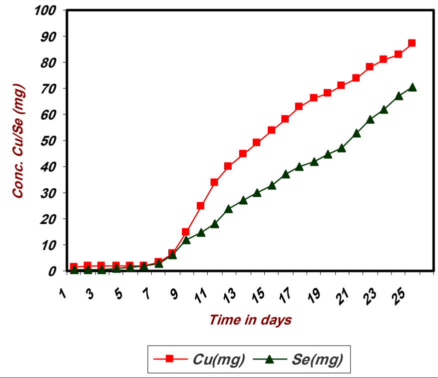
[a] Shake flask leaching of –200 mesh anodic sludge (1g/100 ml HP medium) for 25 days at 32 ºC with 10% inoculum of iron-grow bacteria. Data for *Se-1* from Fig.1.

Final pH values of *Se-1* and *Se-2* flasks were 2.2-2.5. Control (no bacterial inoculation) flask solubilized only 10 mg *Cu* and no *Se* after 25 days and had final pH of 4.3.

[b] Expressed as a ratio of percentages of *Cu* and *Se* extracted from 1 gram- anodic sludge sample (150 mg *Cu* and 100 mg *Se*).

The extraction of *Se* and *Cu* from the anodic slime after 25 days was 43-54 and 52-58%, respectively, of the amount of metal present in the anodic slime.

Figure 1. Time course of shake flask leaching by iron-grown *Se-1* in high-phosphate (*HP*) medium. Conditions are described in Table I.

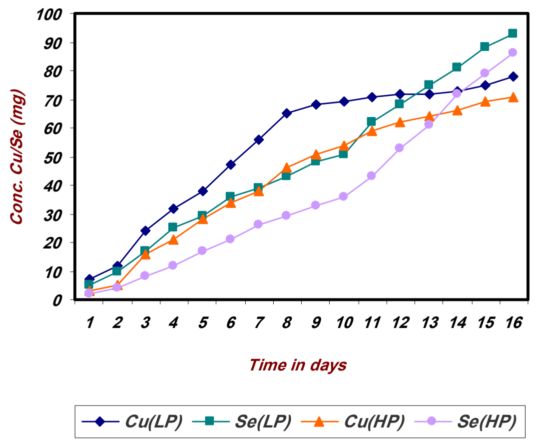


Leaching by Anodic slime Adapted Cells

The effect of anodic sludge adaptation on the leaching activities of the isolates was examined. For this experiment, the T.ferrooxidans isolates *Se-1* and *Se-2* were used both in the HP and LP media.

The T.ferrooxidans isolates required the addition of acid for the first 6 days when growing on the LP medium. The time course leaching experiments in media for T.ferrooxidans *Se-1* is illustrated in Figures 2.

Figure 2. Time course of shake flask leaching by anodic sludge-adapted *Se-1* in low-phosphate(*LP*) or high-phosphate(*HP*) medium.Conditions are described in Table III.



The *Cu* -*Se* ratios were low in spite of adaptation and decreased further during the experiment, more in the LP medium than in the HP medium, indicating a preferential solubilization of *Se* over Cu(Table(2)).

In general, T. ferrooxidans seems to favor the extraction of both *Se* and *Cu* from anodic slime.

Table II. Leaching by anodic sludge-adapted isolates.[a]

Cu-Se Extraction	*Metals extracted (mg/1 g anodic sludge)*								
Bacterial strains	**Cu** *4d*	***8d***	***16d***	**Se** *4d*	***8d***	***16d***	**percentage ratio** *4d*	***8d***	***16d***
(a) In LP medium									
Se-1	32	65	78	25	51	93	1.28	1.27	0.83
Se-2	31	66	73	23	49	90	1.34	1.34	0.81
(b)In HP medium									
Se-1	21	54	71	12	36	85	1.75	1.50	0.83
Se-2	19	53	71	11	34	86	1.72	1.55	0.81

[a] Shake flask leaching of –200-mesh anodic sludge (1 g/ 100ml LP medium or HP medium) for 16 days at 32ºC with 10% inoculum from anodic sludge- grown cells (previous leaching experiment flasks in LP or HP medium after removal of anodic sludge by filtration). In LP medium with Se-1 and Se-2, it was necessary to add to each

flask 70 µL 5M sulfuric acid at inoculation time and every 24h for 3 more days. Final pH values of media were, in LP medium, 2.7(Se-1) and 2.9(Se-2) and, in HP medium, 2.1(Se-1) and 1.9(Se-2).

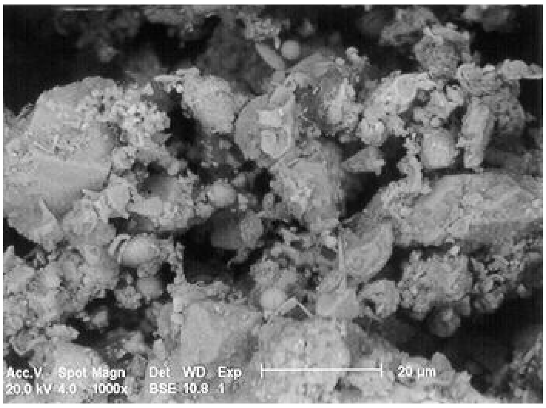


Fig 3. Electron Micrographs of Anodic slime Particles.

CONCLUSIONS

Shake flask Leaching studies of anodic slime containing *CuSe, CuAgSe* and *Ag_2Se* by T. ferrooxidans isolate has produced some interesting conclusions with respect to the leaching ability of each species of bacteria.

The *Cu-Se* extraction ratio by T.ferrooxidans isolates was low and in the range of 1.2.

To pay attention that anodic sludge have *CuSe* (about 87%), we can consider oxidation of this compound by Thiobacillus ferrooxidans.

In Oxidation of *CuSe*, the reactions can, therefore, by expressed as follows

$$CuSe + 2H^+ + \tfrac{1}{2}O_2 \xrightarrow{\textbf{T.ferrooxidans}} Cu^{2+} + Se^o + H_2O \qquad (1)$$

According to this equation, the energy available corresponds to the removal of two electrons from the selenide.

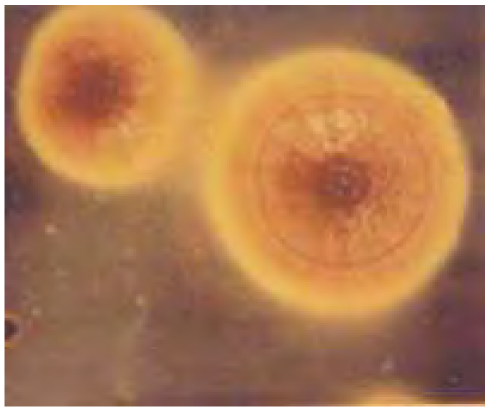
T.ferrooxidans can solublizing selenium to selenite and selenate from anodic slime. It shows that one of the most important reaction in bacterial oxidation is solubilizing selenium from elemental selenium according the following equation:

$$Se^0 \xrightarrow{\textit{\textbf{T.ferrooxidans}}} SeO_3^{2-} + SeO_4^{2-} \qquad (2)$$

The reactions show that conversion of anodic slime to elemental and then conversion of elemental selenium to selenite and selenate are the way of solubilization for selenium from anodic sludge.

Fig 4: *Thiobacillus ferrooxidans.* Isolated from Acid Mine Drainag from Sarcheshmeh copper Industry.

It show that: $Fe^{2+} \longrightarrow Fe^{3+}$



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