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MICROBIAL LEACHING OF IRON, SULFUR AND PYRITE USING *ACIDOTHIOBACILLUS FERROOXIDANS*

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ABSTRACT

In the present study, *Acidithiobacillus ferrooxidans* (allocated from gold and uranium ores of deposit Chantybe in Kazakhstan) was tested for their ability on iron, sulfur and pyrite oxidation during their growth in the media of 9 K and 5K (5K is a medium mdified from 9K, Kamalov, 1990). Results indicated that *A. ferrooxidans* grown in 5 K medium was more active than that grown in 9 K medium and the adapted *A. ferrooxidans* was more active and exhibited a high sulfur oxidation activity than the one which was not adapted.

INTRODUCTION

The autotrophic bacteria *Acidithiobacillus ferrooxidans* are frequently associated with sulfide minerals (Kelly and Wood, 2000), these bacteria obtain their energy from the oxidation of reduced sulfur compounds and also capable of oxidizing iron, this bacterium of the form of a rod is aerobic, acidophilic, autotrophic bacterium, negative in Gram. It is approximately 0.5 μm on width and 2 μm in length. This bacterium is active in pH range of 1.5 to 5. However, its maximum pH is 2 (Holt *et al.*, 1993). *A. ferrooxidans* in this condition it not only use inorganic sulphur structures but also use iron simultaneously as it oxidised the inorganic substrates (Nagaoka *et al.*, 1999 and Tributsch, 2001).

Bacteria *Thiobacillus ferrooxidans* are very widespread in the nature. They are present where the processes of either iron or minerals oxidation take place. They are now the most studied. Besides

Thiobacillus ferrooxidans, *Leptospirillum ferrooxidans* is also widely known, first it oxidises sulphidic and sulfitic ions, bivalent iron, sulfidic minerals of copper, uranium,while some other strains can oxidise pyrite. Bacteria *Sulfobacillus thermosulfidooxidans*, *Thiobacillus thiooxidans*, *T.acidophilus* are rather recently allocated and described, to oxidise S^0, Fe^{2+} and sulfidic minerals. They are also capable to oxidize some representatives of sorts' sulfolobus and acidianus. Among these microorganisms – mesophilic and moderately thermotolerant forms, extreme acidophilic and acidotrophic (Karavaiko *et al.*, 1988).

It becomes a necessity to process low grade ores with complex mineralogy, particularly for base metals including precious and rare earth minerals. Bio-processing techniques offer alternative ways for such complex ores (Van Aswegen *et al.*, 1991).

Additionally, compared to the conventional inorganic reagents such as cyanides, hydrosulfides, dichromate, etc., bacteria are non-toxic and environmentally safe. Many investigations have suggested that certain types of bacterium such as *A. ferrooxidans* may prevent flotation of certain minerals, such as pyrite (Santhiya *et al.*, 2000 and Sharma and Hanumantha, 2001).

The following equations describe the "direct" and "indirect" mechanism for the oxidation of pyrite (Karavaiko *et al.*, 1988):

Direct: $FeS_2+3{,}5O_2+H_2O=FeSO_4+H_2SO_4$ (chemical oxidation) (1)

Indirect:

$2FeSO_4+0{.}5O_2+H_2SO_4=Fe_2(SO_4)_3+H_2O$ (bacterial oxidation) (2)

$FeS_2+Fe_2(SO_4)_3=3FeSO_4+2S$ (chemical oxidation) (3)

$S+1{,}5O_2+H_2O=H_2SO_4$ (bacteria oxidation) (4)

In nature, pyrite is oxidized by oxygen and \ or ferric iron and microbial activity, which can accelerate the rate of this oxidation. A rate law derived by Williamson and Rimstidt (1994) indicates that the abiotic rate of pyrite oxidation increased with increasing oxygen concentration and also increased slightly as pH decreased.

The present work is to syudy the effect of *Acidithiobacillus ferrooxidans* grown in different media (9 k and 5 K) in accelerating the oxidation process of iron and pyrite as well as sulfur oxidation.

MATERIALS AND METHODS

Microorganism and media used

The bacterium used is a strain of *Acidithiobacillus ferrooxidans* isolated from the source of mineral (allocated from gold and uranium ores of deposit Chantybe in Kazakhstan). *A. ferrooxidans* was grown in a Silverman - Lundgren 9K medium (Silverman and Lundgren,1959). The constituents of the mdium are as the following: $(NH_4)_2SO_4$ - 3.0; KCl - 0. /L 1; K_2HPO_4 - 0.5; $MgSO_4 \cdot 7H_2O$ - 0.5; $Ca(NO_3)_2$ - 0.01; $FeSO_4 \cdot 7H_2O$ - 44.2 g pH adjusted by adding 1 m/L H_2SO_4 (0.1N), and the modified medium 5 K by Kamalov (1990) which is consisting of :$FeSO_4 \cdot 7H_2O$ - 25 ; $(NH_4)_2SO_4$ 1.5; KH_2PO_4 - 0.5; $MgSO_4$- 0.5; $Ca(NO_3)_2$ - 0.01 g/L. pH is adjusted by adding 3 ml (conc) H_2SO_4 to adapt the original strain to the S^0 concentrated as a solid substrate, the temperature during leaching was maintained at 30 ± 0.5 °C.

Experiments

1- Oxidation of disulfide iron:

Two hundred fifty ml conical flasks containing 80 ml medium (9K) and others with the medium (5k) each was inoculated with 20 m/L *A. ferrooxidans* to be used in these experiments. Flasks have been placed in a rotary shaker incubator (30°C, 180 rpm).

2- Oxidation of pyrite:

During these experiments 6 flasks were used, two of them contain 80 ml medium (9k) and 20 m/L initial inoculation material *A. ferrooxidans*, the other two flasks contain 80 ml medium (5k) and 20 ml initial inoculation material and the others two flasks contain 100 ml Fe^{+3} (7 g /L). One gram pyrite with a size particle under 0.63 was added to each flask after bacteria oxidized all Fe^{+2} to Fe^{+3}. The flasks were then placed in a rotary shaker incubator at 30°C and 180 rpm.

3- Oxidation of sulfur:

Two sets of conical flasks of 250 m/L in volume containing 20, 30, 50 and 75 m/L. One set is devoted to adapted *A. ferrooxidans* and and the other was devoted to un-adapted one. Both adapted and un-adapted *A. ferrooxidans* were added to the medium 9K without iron to accomblish 100 m/L. All the flasks received pulvorised 1 g sulfur with

a particle size under 0.63 and are deduced on a shaker incubator (180 turns / min) at 30 ± 0.5 °C.

The determination of concentration of Fe^{2+} and Fe^{3+} in solutions was conducted by Trilonmetric method, while the determination of sulfuric acid was done by a volume method (Caloms and Fecenko , ١٩٦٢).

Oxidation-reduction potential and pH were measured by using the pH meter Model– 410 Kavelon.

RESULTS AND DISCUSSION

Disulfide iron oxidation:

Data in Fig. (1) show the activity of Acidithiobacillus *ferrooxidans* against iron oxidation during their growth in the media of 9 K and 5K. Results indicated that *A. ferrooxidans* grown on 5 K medium was more active and exhibited a high disulfide iron oxidation activity than that grown in 9 K medium. However, within 4 days, A. *ferrooxidans* oxidazed all Fe^{2+} to Fe^{+3} in 5K and for 9k in 7 days.

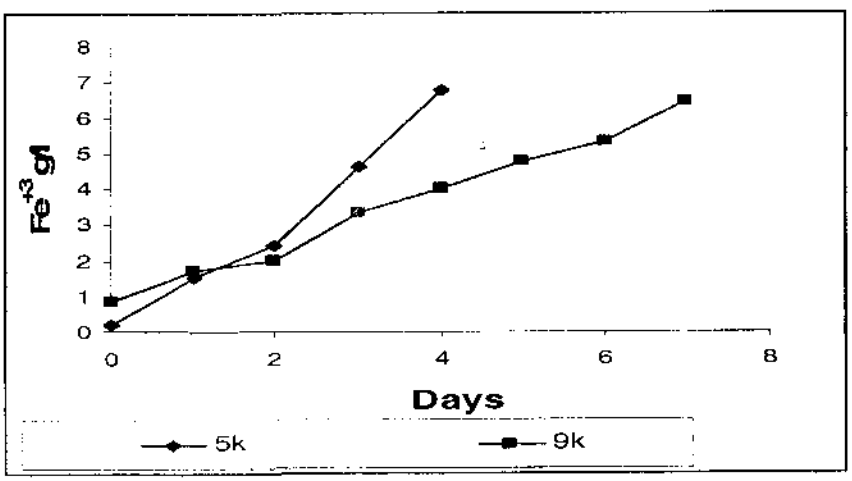

Fig.(1): Concentration of Fe^{3+} during disulfide iron oxidation by *A. ferrooxidans* on medium 5k and 9k.

Pyrite oxidation

Data in Table (1) illustrates the bio-oxidation of pyrite in the media 9K and 5K and in the absence of *A. ferrooxidans* under different incubation periods at the same condition. Results revealed that the rate of dissolution of pyrite, with bacteria cultivated on 5k

medium was higher than that recorded by *A. ferrooxidans* cultivated on 9k medium. The chemical oxidation of Fe^{2+} to Fe^{3+} increased the concentrations with increasing the incubation periods, regardless of bacteria growth medium 9k or 5k. However, the superiority was for the bacteria grown on 5K medium. The incubation period, more than 70 days gave the highest activity in oxidation either with bacteria or with the rate of pyrite leaching due to the bacteria cultivated on the modified 5k medium was higher than the bacteria grown on 9 k medium and ferric solution.

Meanwhile, in the course of bacterial oxidation of pyrite there was an obvious decrease in the environmental pH due to the formation of sulfuric acid (Fig. 2). These results are in agreement with Nordstrom (1982) and Singer and Stumm (1970) who found that in the environment, the rate of sulfide mineral oxidation increased as pH decreased into a range conducive to bacterial mediation of ferrous iron oxidation microbial pyrite oxidation rates begin to exceed chemical oxidation rates at around pH 3.5 - 3. Also, Nordstrom and Ipers (1999) stated that oxidation by ferric iron is roughly two to three orders of magnitude faster than the abiotic oxidation by oxygen at pH 2.

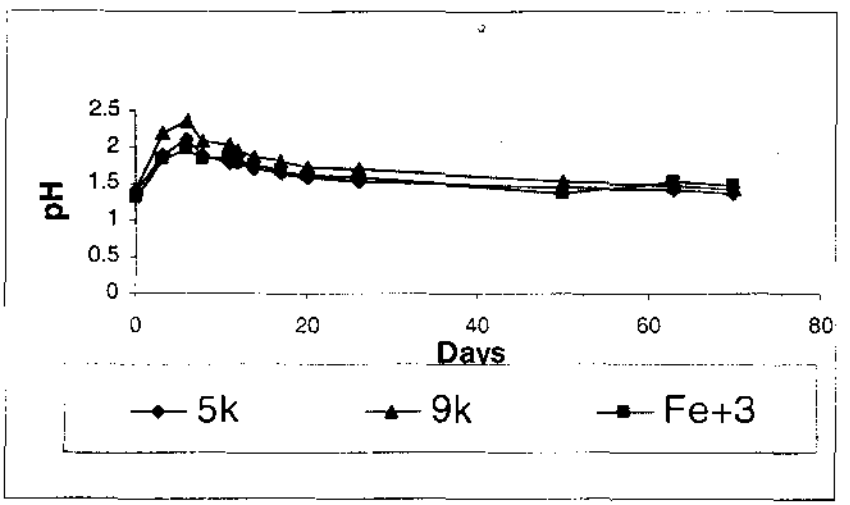

Fig.(2): Chnges in pH during leaching of pyrite in the presence of *A.ferrooxidans* cultivated *in medium 5k*,9K and Fe^{3+} without *A. ferrooxidans*.

Table (1): Leaching of pyrite by *A. ferrooxidans* cultivated in 5K & 9K media with or without ferric solution

Incubation period (Days)	*A. ferrooxidans*						Ferric solution		
	Medium 5K			Medium 9K					
	Fe^{3+} g/L	Fe^{2+} g/L	Fe g/L	Fe^{3+} g/L	Fe^{2+} g/L	Fe g/L	Fe^{3+} g/L	Fe^{2+} g/L	Fe g/L
0	7.00	0.00	7.00	7.00	0.00	7.00	7.00	0.00	7.00
10	7.28	0.00	7.28	7.84	0.00	7.84	6.66	0.56	7.22
20	7.56	0.00	7.56	7.84	0.00	7.84	6.56	0.78	7.34
30	8.40	0.00	8.40	8.00	0.00	8.00	5.88	1.26	7.14
40	10.64	0.00	10.64	9.10	0.00	9.10	6.44	2.24	8.68
50	11.20	0.00	11.20	9.66	0.00	9.66	7.00	3.06	10.06
60	13.44	0.00	13.44	11.20	0.00	11.20	7.56	3.10	10.66
70	14.00	0.00	14.00	12.60	0.00	12.60	7.56	3.52	11.08
80	16.80	0.00	16.80	13.72	0.84	14.56	9.80	4.06	13.86

3- Bio-oxidation of sulfur:

Apparently, Figs. (3) and (4) exhibited that oxidising ability due to the initial *A .ferrooxidans* and its adapted form in their cultures increased with increasing their concentration in cultivation environment. However, the adapted *A . ferrooxidans* form recorded higher oxidation ability for sulfur than un-adapted one due to decreasing pH similar to that noticed by Rawlings (2005).

Elemental sulfur produced in equation [3] may also be re-oxidized to sulphuric acid equation [4] in the presence of sulphur-oxidizing microbes such as *A. thiooxidans* and *A. caldus.*

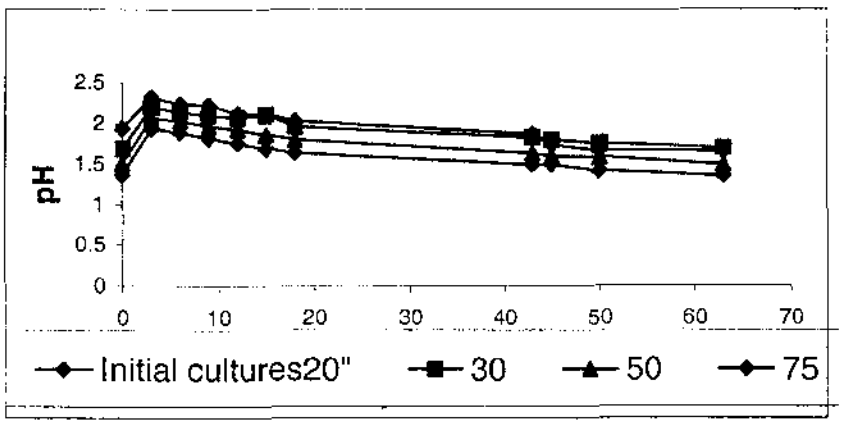

Fig. (3): Changes in pH during oxidation of sulfur in the presence of different concentration *A .ferrooxidans*.

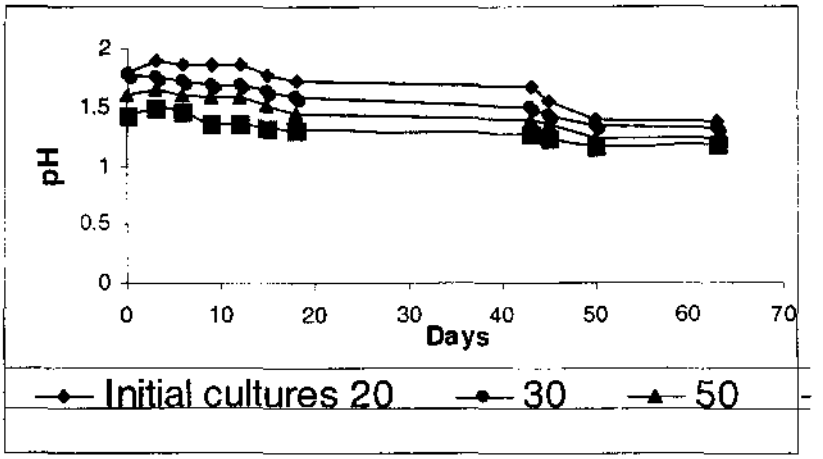

Fig.(4): Changes of pH during oxidation of sulfur in the presence different concentration of adaptation A. *ferrooxidans*.

Table (2): Effect of bacterial densities on the oxidation of sulfur ion

A. erooxidans	Medium 9K mL	Initial culture s mL	Fe^{2+} g/L	Fe^{3+} g/L	pH after 3days	pH after 60 days	Eh after 3days	Eh after 60 days	H_2SO_4 after 60 days g/L
Adaptation	80	20	0.0	1.7	1.89	1.21	313.1	354.1	9.8
Without adaptation	80	20	0.0	1.7	2.32	1.37	288.4	344.6	5.39
Adaptation	75	25	0.0	1.7	1.75	1.22	321.3	353.2	9.8
Without adaptation	75	25	0.0	1.7	2.20	1.52	295.5	336.0	5.3
Adaptation	50	50	0.0	1.7	1.65	1.05	327.1	363.1	14.21
without adaptation	50	50	0.0	1.7	2.07	1.36	302.8	345.2	5.39
Adaptation	25	75	0.0	1.7	1.50	1.05	335.6	367.1	14.7
without adaptation	25	75	0.0	1.7	1.94	1.38	310.3	352.6	7.84

Data in Table (2) reveal that pH decreased during the oxidation of sulphur in the presence of different densities of adapted *A. ferrooxidans* more than in the presence of different densities of *A.ferrooxidans*. In this respect, the presence of either adapted or not adapted *A. ferrooxidans* with different densities increased the oxidation reduction potential (Eh) and the formation of H_2SO_4. Also, Oxidation of the restored iron (Fe^{2+}) and sulfurs (S) in usual sulfide, pyrite (FeS_2), leads to formation of strong sulfuric acid (H_2SO_4). Kawatra and Natarajan (2000) mentioned that bacterial oxidation of sulphide ores using chemoauto-trophic bacteria such as *Thiobacillus. ferrooxidans* and *T. thiooxidans* is a well-known process in bio-hydrometallurgy, and thought to occur by a combination of direct and indirect mechanisms. These bacteria attach themselves to the sulphide ore particles and from etch patterns on the mineral surface.

As a result of *A. ferrooxidans* activity in oxidation of a considerable part of iron sulpfide, other connections of sulfur and sulfide minerals, the activity of the *A.ferrooxidans* took place to be higher effective in medium 5K than in medium 9k and chemical oxidation. Adapted inoculum has currently possessed more bio-oxidation of sulfur. However, it could be concluded that it is possible to use bio-oxidation of ore as pre-treatment by *A.ferrooxidans* cultured in medium 5K not only to increase the final gold recovery but also to recover other available metals contained in the ore.

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الاستخلاص الميكروبي للحديد والكبريت ومعدن البيريت باستخدام *Acidithiobacillus ferrooxidans*

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إن النشاط الميكروبي للبكتريا اسيدوباسيلس فيرواوكسيدنز للمادة الخام للذهب واليورانيوم (شا نتو بى - كازاخستان) التي تؤكسد جزء من كبريتيد الحديد وبعض العناصر الاخرى لعنصر الكبريت ، يكون بمعدل أعلى في البيئة (ك ٥) عند مقارنته في البيئة (ك ٩) وايضا بالاكسدة الكيميائية.ونجد ان البكتريا اسيدوباسيلس فيرواوكسيدنز المؤقلمة تقوم بأكسدة الكبريت اكثر من البكتريا الغير مؤقلمة. وبذلك من الممكن استخدام الاكسدة الميكروبية للبكتريا اسيدوباسيلس فيرواوكسيدز للمادة الخام في بداية الاستخلاص للمعادن النفيسة الامر الذي يؤدي لزيادة الناتج النهائي لعنصر الذهب وايضا امكانية الحصول على العناصر الاخرى المختلطة بالمعدن الخام.