Fe Extraction from Çayeli Copper Ores by Bioleaching with Eco Freiendly *Acidithiobacillus ferrooxidans*

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Summary: Recently, biological treatment; an important recovery process, has became important from the environmental and economical respects in recovery of metallic values from low-grade sulfur-bearing ores or concentrates. Bacterial ore leaching can be applied to extract heavy metals from low grade ores, industrial wastes and other materials on an industrial scale by different procedures. The main objective of this work was to investigate the dissolution of Fe from Çayeli copper ores, via a bioleaching process using *Acidithiobacillus ferrooxidans*. Experiments performed with batch operation in jar test equipment were conducted at different pH values, pulp densities, inoculum volumes, particle sizes, stirring conditions and operation times. The optimal parameters were found as follows; at pH 2, the pulp density; 4% (w/v), inoculum volume; 4% (v/v), stirring rate; 120 rpm and particle size; -0.053 mm for 192nd and 288th hours, at pH 2, the pulp density; 4% (w/v), inoculum volume; 5% (v/v), stirring speed; 200 rpm and particle size; -0.053 mm for 384th and 480th hours. By performing the bioleaching process under these conditions, almost 99% of the iron extent in the ore was transfer from ore into solution, however the experiments in which distilled water was used instead of modified 9K*, only 18.5% Fe efficiency was obtained.

Keywords: Bioleaching, *Acidithiobacillus ferrooxidans*, Extraction, Copper, Iron, Optimization.

Introduction

Bioleaching is a new technique used by the mining industry to extract minerals such as gold and copper from their ores while traditional extractions involve many expensive steps such as roasting and smelting, which requires sufficient concentrations of elements in ores [1, 2]. In general, bioleaching is a process described as "the dissolution of metals from mineral source by certain naturally occurring microorganisms" or "the use of microorganisms to transform elements so that the elements can be extracted from a material when water is filtered trough it" [3]. The majority of the microorganisms used for bioleaching are autotrophic aerobes; therefore carbon dioxide and oxygen are the essential nutrients for their growth and survival, their adequate supply must be ensured in order for the oxidation process to be successful [4]. In these processes, bacteria catalyze the dissolution of metals from minerals. Therefore, Lacey and Lawson [5] showed that bacterial oxidation of ferrous iron is about 10^5-10^6 times faster than the chemical oxidation of ferrous iron in the range of pH 2-3. By far the most important group of bacteria which are involved in leaching of sulphide minerals are the acidophilic *Thiobacilli* which form part of the family *Thiobacteriaceae.* This species does not only utilize inorganic sulphur compounds and ferrous ion simultaneously can oxidize inorganic substrates. *Acidithiobacillus ferrooxidans* is an aerobic, acidophilic autotrophic, gram-negative bacterium. This bacterium is active over a pH range of 1.5-5 and with optimum growth at pH 2.0 [6]. The use of these microorganisms for bioleaching of chalcopyrite requires the knowledge of the mechanisms involved in the process. Initially, chalcopyrite can be oxidized by dissolved oxygen according to equation 1, favored for acidic solution. As known, two modes of bacterial attack can be distinguished: In indirect attack (Equation 3), the role of bacteria is to regenerate the oxidant ferric ion in the bulk phase using the ferrous iron from the chemical oxidation of the metal sulphide in the ore by ferric iron (Equation 3 and 4). In the direct attack (Equation 1 and 2), the bacteria cause to dissolution of the metal sulphide by attaching to the mineral surface and oxidizing it enzymatically by conveying electrons from the reduced moiety of the mineral. Some authors (Sand *et al*., 2001) suggested that because Fe^{3+} oxidizes metal sulphide with both the direct and indirect mechanisms, there is no difference between the two mechanisms [7]. Their model emphasizes a similarity in the chemistry of attack of the sulphide moiety by iron, and makes no distinction between ferric iron in the bulk phase and ferric iron bound in the cell envelope [8-11]

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$$CuFeS_2 + O_2 + 4H^+ \xrightarrow{bacteria} Cu^{2+} + Fe^{2+} 2S^0 + 2H_2O \quad (1)$$

$$CuFeS_2 + 4Fe^{3+} \xrightarrow{bacteria} Cu^{2+} + 5Fe^{2+} 2S^0 \quad (2)$$

$$4Fe^{2+} + O_2 + 4H^+ \xrightarrow{bacteria} 4Fe^{3+} + 2H_2O \quad (3)$$

$$S^0 + \frac{3}{2}O_2 + H_2O \xrightarrow{bacteria} H_2SO_4 \quad (4)$$

During the last 30 years bioleaching of minerals has opened up new opportunities for extractive metallurgy and biohydrometallurgy is now practiced in the copper industries, especially for the treatment of low-grade ores. As a result, in the last 20 years, 10 bio-heap leaching plants, 7 bio-reactor plants for sulphidic concentrate and countless pilot scale operations have started worldwide. Conventional mineral processing of complex sulphide ores, carried out by differential flotation, often produces a bulk concentrate that is difficult to process. But bio-reactors open up a promising future. The only commercial scale stirred tank reactor process for Cu–Pb–Zn concentrate is running at Olympia Greece with a daily throughput of 0.048 ton/m^3 day [12]. The potential for recovering zinc by bioleaching of Moore cake using *At. ferrooxidans* bacteria (some authors) studied to assess [13]. The oxidation of ferrous iron and elemental sulfur by *At. ferrooxidans* that was absorbed and unabsorbed onto the surface of sulfur prills was studied [14]. Another work was to evaluate a bioleaching process from the treatment of two concentrates, (i) a copper gold bearing concentrate and (ii) an ordinary copper flotation concentrate, using mesophilic bacteria [15]. The aim of this paper is to investigate the extraction of the Fe from the sulphidic complex Cu-Zn-Pb ores and to determine to the optimum parameters.

Experimental

Ore samples

The complex sulfidic Cu-Zn-Pb ore used in the experiments was obtained from Rize-Çayeli-Madenköy region in Turkey. The composition of this ore called "klastik ore" by authorized of Company was consisted of pyrite, chalcopyrite, galena, sphalerite, tetrahedrite, markasite bornite [16, 17]. The natural ore was crushed to obtain a sample in the size range of 1 mm by using BB2, Masch Nr 65110 jaw crusher. After crushed ore was ground by Retsh, SK100 Standard Guβeisen mill, it was sieved by using 0.125 mm, 0.090 mm and 0.053 mm - ASTM standard sieves. The ore samples were analyzed in Laboratory of Çayeli Copper Organisation Corp., Rize, Turkey (Table-1) and Laboratory of The Unit of Analytical Chemistry, Mineral Research and Exploration Institute, Ankara (-2).

Table-1: Full analysis of the ore from Çayeli-Madenköy in Turkey**.

elements	%	ppm	elements	%	ppm
Cu	4.18		Na		0.04%
Zn	11.33		MgO	0.43	
Pb	0.41		Sb		<25
Fe	27.94		Bi		44.0
Total	43.9		Cr		<25
S	37.30		Co		<10
Ag	96.0 (g/t)		Ge		<30
Au	1.6 (g/t)		Mn		0.08%
Ba	1.79		Hg		6.0
Cd	0.05		Mo		<25
As	0.16		Ni		<10
Al_2O_3	1.18		K	0.11	
CaO	0.63		Se		105.0
SiO_2	5.65		Te		17.0
Cl	<50		Tl		57.0
F		192.0	Sn		<25
C	0.28		Ti		<25
			V		<25

** Laboratory of Rize-Cayeli-Madenkoy Copper Organisations

Table-2: The chemical analysis of the four particle sizes of the ore from Çayeli-Madenköy in Turkey*.

PARTICLE SIZE (mm)	Cu (%)	Zn (%)	Pb (%)	Fe (%)	Au (g/t)	Ag (g/t)
+0.125	2.95	8.42	0.53	31.78	1.5	82.5
-0.125+0.090	3.76	13.02	0.43	27.81	1.6	109.7
-0.090+0.053	4.14	14.52	0.23	24.30	1.9	108.1
-0.053	4.80	15.80	0.58	20.99	1.8	111.2

* Laboratory of the Unit of Analytical Chemistry of M.T.A.

The Preparation of Media

Four species of media were prepared by dissolving various amounts of NH_4Cl, KCl, NaCl, $MgCl_2.6H_2O$, $CaCl_2.2H_2O$, $FeSO_4.7H_2O$, $(NH_4)_2SO_4$, K_2HPO_4, $MgSO_4.7H_2O$ and $Ca(NO_3)_2$ in 1000 mL distillated water. In some experiments, the grown ore was used instead of $FeSO_4.7H_2O$, and KH_2PO_4 instead of K_2HPO_4. Ingredients of these media are given in Table-3. All the chemicals were supplied from Merck.

Table-3: Various 9K media for bacteria growth in isolation and their ingredients [19].

Nutrıents	$9K^{(1)}$	Modıfıed $9K_{33}$	Modıfıed 9K33*	MODIFIED 9K*
NH_4Cl (g)	0	0	0	0
KH_2PO_4 (g)	0	0.1	0.1	0.1
KCl (g)	0.1	0.1	0.1	0.1
NaCl (g)	0	0	0	0
$MgCl_2.6H_2O$ (g)	0	0	0	0
$CaCl_2.2H_2O$ (g)	0	0	0	0
$FeSO_4.7H_2O$ (g)	14.7	33.0	15.0~33.0	0
$(NH_4)_2SO_4$ (g)	3.0	3.0	3.0	3.0
K_2HPO_4 (g)	0.5	0	0	0
$MgSO_4.7H_2O$ (g)	0.5	0.5	0.5	0.5
$Ca(NO_3)_2$ (g)	0.01	0.01	0.01	0.01
Ore (g)	0	0	1-5	1-5
pH	2.0-2.3	2.0-2.3	2.0-2.3	2.0-2.3
Distillated water	1000 mL	1000 mL	1000 mL	1000 mL

Microorganisms

Bacteria used in the all experiments were obtained from AMD samples of Artin-Murgul-Damar

Copper Mine. This bacterium is *At. ferrooxidans*, which is characterized by its chemosynthetic metabolism and its compounds in order to produce energy for growth. *At. ferrooxidans* is aerobic, acidophilic autotrophic, non-spore forming, Gram-negative, motile and single-pole flagellated bacterium. It is a rod-shaped bacterium approximately of 5 μm in width and 2 μm in length (Fig. 1). This bacterium is active over a pH range of 1.5-5.0 with optimum growth at pH 2.0. It requires a source of nitrogen, phosphate and trace amounts of calcium, magnesium and potassium. Energy for growth is obtained from the oxidation of ferrous iron, insoluble sulphides, and soluble sulphur compounds. Most thiobacilli are using the CO_2 from the atmosphere as carbon source for synthesis of new cell materials [6-18, 19].

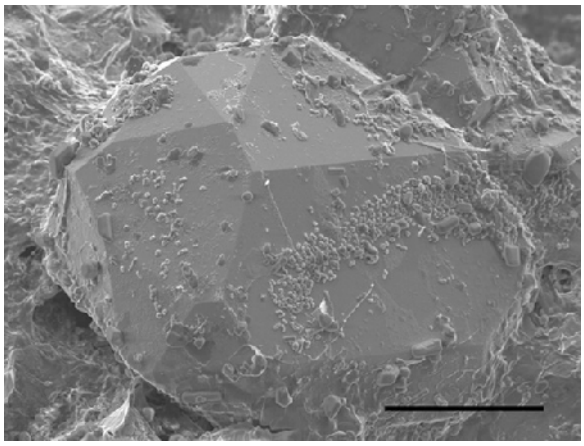


Fig. 1: SEM (scanning electron micrograph) of A. ferrooxidans, adhering on the surface of a Pyrit crystal. Bar: 20 μm[22] (By Thomm, M.)

Bacterial Inoculum (Cultivation, Isolation-Purification and Adaptation)

These experiments were carried out in order to obtain bacteria with enough population and enough purity for bioleaching.

Cultivation of Bacteria: For this purpose, 100 mL 9K medium and 10 mL AMD sample were mixed in 250 mL - erlenmayer flasks during the first 13 days, using a ROSI 1000 Thermolyne Orbital Shaking Incubator. Along this time, color changing of solution were observed and noted, Fe(II) and Fe(III) concentrations were analyzed periodically and bacteria's population were counted or observed microscopically.

Isolation-Purification of Bacteria: For this purpose, 17 experiments following each other carried out by combinations of different media. In the first experiment, 5 mL of bacteria variety(r) from cultivation and 100 mL 9K medium were incubated by mixing in a 250 mL-erlenmayer flask at 23-28°C and shaking speed of 110 rpm during 10 days. Then, isolation of bacteria was carried out by using various volumes of bacteria varieties (from 5 mL to 1.0 mL) obtained from preceding experiment and various volumes of 9K, 9K* and $9K_{33}$ media in the same way [17-24]. Cultivation and isolation-purification levels are given in Table-4.

Purification of bacteria were performed with 9K, 9K* and $9K_{33}$ media with agarose concentration at pH 2.00 in sterile Petri's dishes according to Manning's method [17-23, 24]. After four or five isolation experiments, serial dilutions of the enrichment cultures at exponential growth phase were made using iron-free 9K medium at pH 2.0. In the result of these procedures, ferrous and ferric ion concentrations were evaluated and color changes in the solutions were watched periodically. During this time, color of the solution was invigilated to turn russet from transparent. Periodically, the grown bacteria were transferred in definite portions from their medium to a new medium and in same way, the new strains and subcultures were formed. Isolation and purification experiments were continued until the bacteria reached enough purity.

Adaptation of bacteria: Besides, the process of adaptation to the ore was also made at the same time. For this reason, definite portions of the ore which will be used in bioleaching experiments were added to the medium instead of $FeSO_4.7H_2O$ added to $9K_{33}$ at the end of the each 4^{th} or 5^{th} inoculation, and this new medium was named by "modified 9K*" [8, 23, 25, 26]. Meantime, the evolution of color of the solution was watched; ferrous and ferric analyses and microscopic examination were made and noted. After the bacteria were grown in this medium, they were transferred to the other fresh medium and the inoculation was continued until the bacteria adapted to the ore.

Counting of Bacteria: The growth of strains obtained by the isolation - purification experiments were measured by cell count (with 'Cell Counts by Hemocytometer') (1/400 mm^2-1/25 mm^2) of the supernatants of the suspensions, microscopically [27, 28].

Bioleaching Experiments: The parameters selected at the bioleaching experiments are pH, pulp density (percent of ore in bioleaching solution), inoculum volume (volume of solutions of bacteria), particle size and stirring speed [22, 29, 30]. Levels of parameters are given in Table-5.

Table-4: The progress of isolation of the strain of bacterium-(*r4A1FC2B3*).

Inoculatıon No	Subculture of Bacterıum	Medıum Name	Contents of Medıum	Condıtıons Of Isolatıon			
				Stırrıng Tıme (day)	pH	Stırrıng Speed (rpm)	Temperature of Medıum (°C)
1	-	R	(100 mL 9K+10 mL AMD*)	13	2.5	115	23-28
2	*r*	R_1	(100 mL 9K+5 mL R)	10	2.5	115	23-28
3	*r1*	R_2	(100 mL $9K_{33}$+5 mL R_1)	13	2.5	115	23-28
4	*r2*	R_3	(100 mL $9K_{33}$+3 mL R_2)	13	2.5	115	23-28
5	*r3*	R_4	(100 mL $9K_{33}$+1 mL R_3)	16	2.5	115	23-28
6	*r4A*	R_5	(100 mL $9K_{33}$+5 mL R_4)	13	2.3	115	23-28
7	*r4A*	R_{13}	(100 mL $9K_{33}$+5 mL R_5)	14	2.11	115	23-28
8	*r4A1*	R_{10}	(100 mL $9K_{33}$+1 mL R_{13})	15	2.05	115	23-28
9	*r4A1*	R_{11}	(100 mL $9K_{33}$+1 mL R_{10})	23	2.05	115	23-28
10	*r4A1*	R_{12}	(100 mL $9K_{33}$+1 mL R_{11})	22	2.06	115	23-28
11	*r4A1F*	R_{19}	(200 mL $9K_{33}$+2 mL R_{12})	27	2.06	115	23-28
12	*r4A1FC*	R_{24}	(150 mL $9K_{33}$+2 mL R_{19})	27	2.06	115	23-28
13	*r4A1FC1*	R_{25}	(150 mL $9K_{33}$+1.5 mL R_{24})	38	2.06	115	23-28
14	*r4A1FC1*	R_{38}	(150 mL $9K_{33}$+1.5 mL R_{25})	34	1.95	100	23-28
15	*r4A1FC2*	R_{39}	(200 mL $9K_{33}$+2.5 mL R_{38})	33	2.00	100	23-28
16	*r4A1FC2*	R_{44}	(200 mL $9K_{33}$ + 2.5 mL R_{39})	26	2.03	110	23-28
17	*r4A1FC2B*	R_{50}	(150 mL $9K_{33}$ + 1.5 mL R_{44})	13	2.00	120	23-28
18	*r4A1FC2B*	R_{50}	(150 mL $9K_{33}$ + 1.5 mL R_{44})	20	2.00	100	23-28

(*) AMD sample of Artvin-Murgul-Damar Copper Mine

Table-5: Parameters and their levels.

	Parameters	Levels			
		1	2	3	4
A	pH →	1.50	2.00	2.25	2.50
B	Pulp content (%)(w/v) →	4	6	8	10
C	İnoculum volume (%) (v/v)→	2	3	4	5
D	Stirring speed (rpm) →	90	120	150	200
E	Particle size (mm) →	+0.125	-0.125+0.090	-0.090+0.053	-0.053

Table-6: [L_{16}=(4^5)] randomize experimental plan table.

Symbol	(A)	(B)	(C)	(D)	(E)
	pH	Pulp Content (%)	Inoculum Volume (%)	Stirring Speed (rpm)	Particle Size (mm)
1→	1.50	4	2	90	+0.125
2→	1.50	6	3	120	-0.125+0.090
3→	1.50	8	4	150	-0.090+0.053
4→	1.50	10	5	200	-0.053
5→	2.00	4	3	150	-0.053
6→	2.00	6	2	200	-0.090+0.053
7→	2.00	8	5	90	-0.125+0.090
8→	2.00	10	4	120	+0.125
9→	2.25	4	4	200	-0.125+0.090
10→	2.25	6	5	150	+0.125
11→	2.25	8	2	120	-0.053
12→	2.25	10	3	90	-0.090+0.053
13→	2.50	4	5	120	-0.090+0.053
14→	2.50	6	4	90	-0.053
15→	2.50	8	3	200	+0.125
16→	2.50	10	2	150	-0.125+0.090
Opt. parameter →	2.00	4	5	120	-0.053
Control →	2.00	4	5	120	-0.053

In order to obtain the optimum parameters, the experiments were carried out with experimental plan prepared according to Taguchi method and given in Table-6. The change with the time of parameters used in the experiments was considered and the data till 96th hour were not used because bioleaching was not expected.

Bioleaching experiments were carried out in 1000 mL-beakers containing 500 mL of iron-free 9K medium with different portions of bacteria solutions by using two jar test apparatus, marked Velp Scientifica, Leaching test/Jar Test JLT6. The measurements of pH and temperature in solutions were performed by Schott Instruments Handylab Multi 12 pH-Meter.

In order to observe the effects of noise sources on the bioleaching process, each experiment was repeated twice under the same conditions and at different times. At the end of the bioleaching experiments, the samples were analyzed once 96 hours during 20 days.

The bacteria used for each bioleaching experiment were enriched before 10 days from the bioleaching experiments, and then the suspension containing maximum concentration-cell was used as inoculum in the bioleaching experiments. 20 days-

each experiment was repeated twice. Then the confirming experiments were carried out twice with the obtained optimum parameters. The control experiments were also carried out with optimum parameters using distillated water instead of 9K medium.

In order to determine the optimum parameters, bioleaching experiments were carried out at 1.5, 2.0, 2.25 and 2.50 pH within possibility limits necessary for bacterial oxidation[29]. During the experiments, the measurements of temperature and pH were routinely made at 24^{th}, 48^{th}, 96^{th}, 264^{th}, 288^{th}, 384^{th}, 432^{nd} and 480^{th} hours. At the end of each measurement, increase or decrease in pH were adjusted with 10%(w/w) H_2SO_4 or 0.5 M NaOH, respectively. The temperatures were generally kept in 23-28 ºC. In general, the pH values increased rapidly at first 24 hours, but it was adjusted with sulphuric acid to the initial values. The pH value did not change up to 300^{th} hour, but, it decreased to the bottom of the initial value after the 300^{th} h. Changes of pH were very small at the 1.5 and 2.00 pHs, but, the rises or falls in pH were much at the 2.25 and 2.50 pHs. This behavior is due to the adaptation of the bacteria at the pH=2.0 during the isolation and growth. The adaptation stage of the bacteria was much more short time at pH≤2.00, but the adaptation time increases over this value.

Selected parameters and their levels were kept constant for each experiments and the bioleaching was carried out during the 480 hours. Before the determining of the optimum parameters, the relations between Fe efficiencies obtained at the bioleaching experiments and parameters were investigated.

Analytical Determinations: Fe(II) and Fe(III) concentrations in the solution were analyzed during both bacteria isolation and bioleaching experiments. The concentrations of ferrous ions were determined by spectrophometry and the concentration of ferric ions by titration method with EDTA [17-21-31-32-33]. Disodium EDTA, 5-sulphosalicylic acid, ammonium chloride, 1,10-phenanthroline, ammonium acetate, potassium permanganate, acids and bases supplied from Merck were used in preparation of the analyses solutions. For analysis of Fe(II), 0.5 mL sample was mixed with 20 mL of 0.1% -1,10- phenanthroline and 10 mL of ammonium acetate tampon solution prepared by adding 250 g ammonium acetate and 700mL concentrated acetic acid to 150 mL distilled water and Fe(II) concentration was read from spectrophotometer. For Fe(III) analysis, 2 mL sample was mixed 1 mL 5% 5-sulphosalicylic acid and titrated with 0.01M Titriplex III solution at 2-3 pH.

Results and Discussion

*Bacterial Inoculum***:** The AMD samples which the strain *r4A1FC2B3* was isolated were selected from acidic deposit water of Damar Mine in Murgul, Turkey. Acidophilic bacteria which live around this region were continuously subject to the changes of seasonable temperature, different solute concentration and ore which would be used in the bioleaching experiments and they had adapted to these conditions. Because of these properties AMD samples were selected from this region. These properties also caused to increasing of the metal extraction. Besides, the optimum pH of culture medium was generally about 2.3. In opposition to this, isolated species grew rapidly at the lower pH values (2.00-2.25). This situation was noted at the study of [23]. *A.f.* used the bioleaching experiments were isolated from these AMD samples.

At the end of the 192^{nd} hour, it was decided that for both maximum bacterial activity and maximum cell numbers, optimum age of bacteria which will be used in the bioleaching experiments was 10-11 days. In the longer times, both bacterial activity and cell numbers decreased, and also, jarosite formation was seen in the solution. The population of the obtained strains was about $2.08x10^6$ cells/mL at the end of 10-11 days, counting by Cell Counts by Hemcytometer. Besides, microscopic examination of *A.f.* isolated from solid and liquid media showed that these microorganisms were single or in pairs rod shape cells and any organism except these bacteria was not present. The colonies of strain *r4A1FC2B3* became brown in one week. In Lavalle's study [23], this time had been found two weeks. It was seen that this strain had a higher ability of oxidizing ferrous [23].

Ferrous and ferric ion concentrations were periodically analyzed to follow the growth of strain of bacterium-(*r4A1FC2B3*) which will be used in bioleaching experiments (Table-7). As a result of the analyses, ferrous quantity was 6607.99 mg/L in the modified $9K_{33}$ containing 33 g $FeSO_4.7H_2O$ in the starting. The bacteria oxidized ferrous to ferric in the medium during bacterial growth. The quantities of ferrous and ferric were determined at the end of a 192 h-incubating experiment and were evaluated. According to this, the growth of *A.f.* was separately examined in the two different shaken flasks and it is determined that the Fe(III) quantity in the solution was 98.8% of total iron oxidized by the bacteria at the end of the 192^{nd} hour. The evolutions of the ferrous and ferric concentrations in the solution

indicated a bacterial activity [15]. Besides, the change of the color of solution was due to the bacterial activity. It was determined by the observations that while the color of the solution was transparent at the beginning, the color transformed into the straw colored at the end of the 96th hour, into the gamboge at the end of the 168th hour and then also into the dark russet at the end of the 192nd hour, respectively. In the result, ferrous concentrations decreased, ferric concentrations increased, and the bacterial activity was at maximum level. Thus, the number of bacteria in solution increased steadily up to the first 80-96 hours of the experiment.

Table-7: Fe analysis of the strain of bacterium-(*r4A1FC2B3*).

Strain of Bacteria	Time (h)	Fe(II) mg/L	Fe(III) mg/L	Total Fe mg/L
r4A1FC2B3-1	48	53.68	1045.32	1099.00
	96	137.13	1460.77	1597.90
	168	135.03	4529.74	4664.77
	192	29.09	6452.87	6481.96
r4A1FC2B3-2	48	53.69	904.61	958.30
	96	137.13	1427.27	1564.40
	168	135.03	4502.94	4637.97
	192	25.49	6506.48	6531.97

Bioleaching: Bioleaching is a simple and effective technology for metal extraction from low-grade ores and mineral concentrates [18]. Bioleaching is depended on ferric concentration which is produced by bacterial oxidation of the ferrous as in (equation 3) [9]. *A.f.* oxidizes the sulphidic ores with direct bonding and also, is able to oxidize ferrous ion to ferric ion [8-10].

The effect of the bacteria on the dissolution of pyrite could be determined directly by comparing the amounts of iron released with and without bacteria [34]. By considering this situation, a complex sulphidic Cu-Pb-Zn ore was selected for the bioleaching experiments in this study [21-31, 17]. In the experimental procedure, continuous jar tests were carried out for providing of the iron dissolution. During these experiments, firstly, different parameters were worked, the optimum conditions were determined, and total Fe, Fe(II) and Fe(III) in the solution were analyzed. After the iron dissolution was provided, bounded Fe in the ore was transferred into the solution as Fe(II), transferred Fe(II) was oxidized to Fe(III) by bacteria, pyrite and chalcopyrite were dissolved with ferric iron. Sulfur compounds were also oxidized to sulphate by bacteria during the bacterial oxidation and a part of this passed into the solution and another part also precipitated with Pb^{2+} ions in solution as $PbSO_4$ (Equation 5 and 6).

$$S^{=} + 2O_2 \xrightarrow{\text{bacteria}} SO_4^{2-} \quad (05)$$

$$Pb^{2+} + SO_4^{2-} \rightarrow PbSO_4^{2-} \quad (06)$$

The iron concentration values used in the determination of the optimum parameters were arithmetical average of total Fe results of three times repeated experiments. These values are given in Table-8 and Fig. 2.

Table-8: Mean average of Fe efficiencies.

Experiment No	Total Fe efficiency (%-average)			
	t=192 h	t=288 h	t=384 h	t=480 h
1	4.70	7.15	7.65	10.40
2	4.45	5.50	6.30	7.05
3	4.95	5.95	7.70	10.25
4	9.20	17.20	27.7	43.95
5	31.30	58.45	79.85	97.35
6	13.10	32.25	39.65	55.45
7	4.90	6.35	6.75	13.85
8	5.70	6.10	8.85	11.55
9	12.25	27.75	34.7	43.00
10	2.10	2.35	4.60	5.00
11	24.10	27.35	35.5	49.05
12	3.95	5.40	7.20	17.85
13	28.15	41.50	46.05	56.30
14	13.00	15.85	18.10	39.85
15	1.90	2.10	3.65	4.35
16	1.00	1.65	2.15	2.85
average	10.30	16.43	21.02	29.26

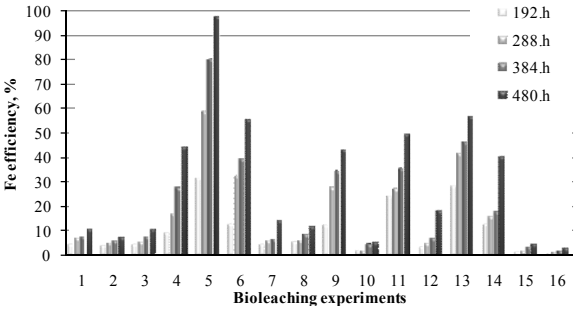
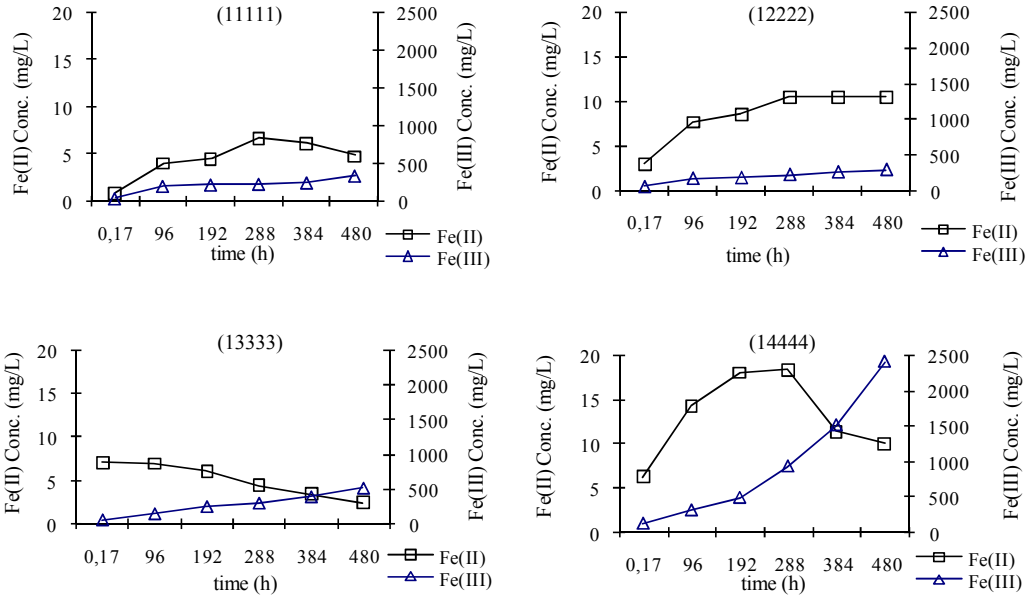
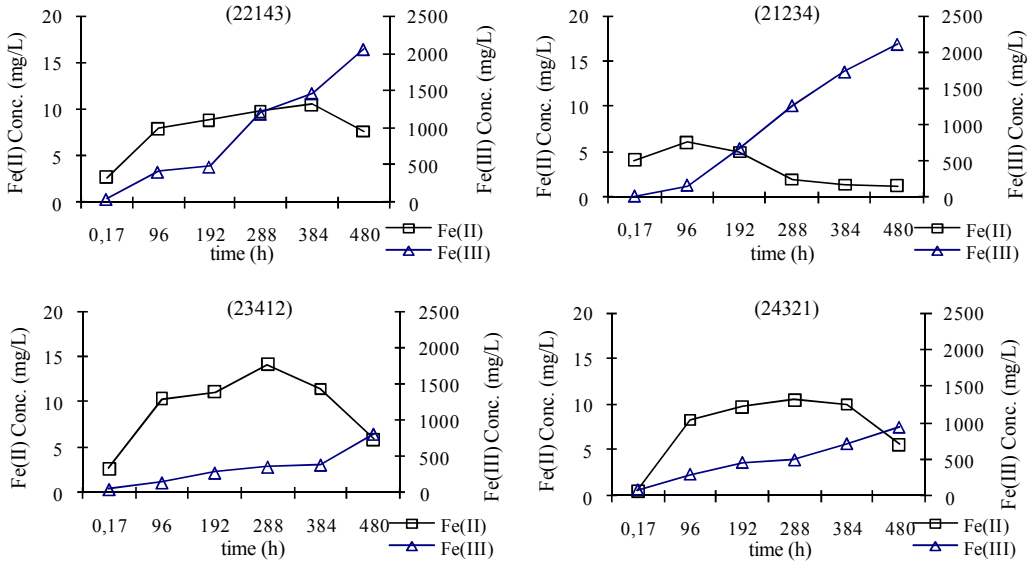


Fig. 2: The mean average of Fe efficiencies in the bioleaching experiments.

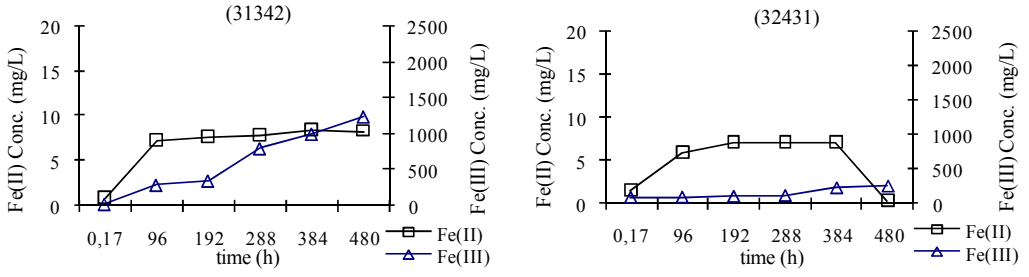
Determination of optimum parameters: At pH 1.50, the highest total iron efficiency was found at the experiment1 in which (v/v) inoculum volume was 5%, pulp density 10% (w/v), stirring speed 200 rpm and particle size -0.053 mm, respectively. In the experiments which pH was kept constant at 2.00, the highest total iron efficiency was found at the experiment 5 under which the conditions were 3% (v/v) inoculum volume, 4% (w/v) pulp density, 150 rpm stirring speed and -0.053 mm particle size. In experiments which pH was kept constant at 2.25, the highest total iron efficiency was found at the experiment11 under which the conditions were 2% (v/v) inoculum volume, 8% (w/v) pulp density, 120 rpm stirring speed and -0.053 mm particle size. In the experiments which pH was kept constant at 2.50, the highest total iron efficiency was found at the experiment13 under which the conditions were 5% (v/v) inoculum volume, 4% (w/v) pulp density, 120 rpm stirring speed and -0.090+0.053 mm particle size. Variation of the Fe(II) and Fe(III) concentrations for four various pH were given in Fig. 3.


(11111)
(12222)
(13333)
(14444)
Fe(II) Conc. (mg/L)
Fe(III) Conc. (mg/L)
time (h)
0,17 96 192 288 384 480
Fe(II)
Fe(III)

(a)


(22143)
(21234)
(23412)
(24321)
Fe(II) Conc. (mg/L)
Fe(III) Conc. (mg/L)
time (h)
0,17 96 192 288 384 480
Fe(II)
Fe(III)

(b)


(31342)
(32431)
Fe(II) Conc. (mg/L)
Fe(III) Conc. (mg/L)
time (h)
0,17 96 192 288 384 480
Fe(II)
Fe(III)

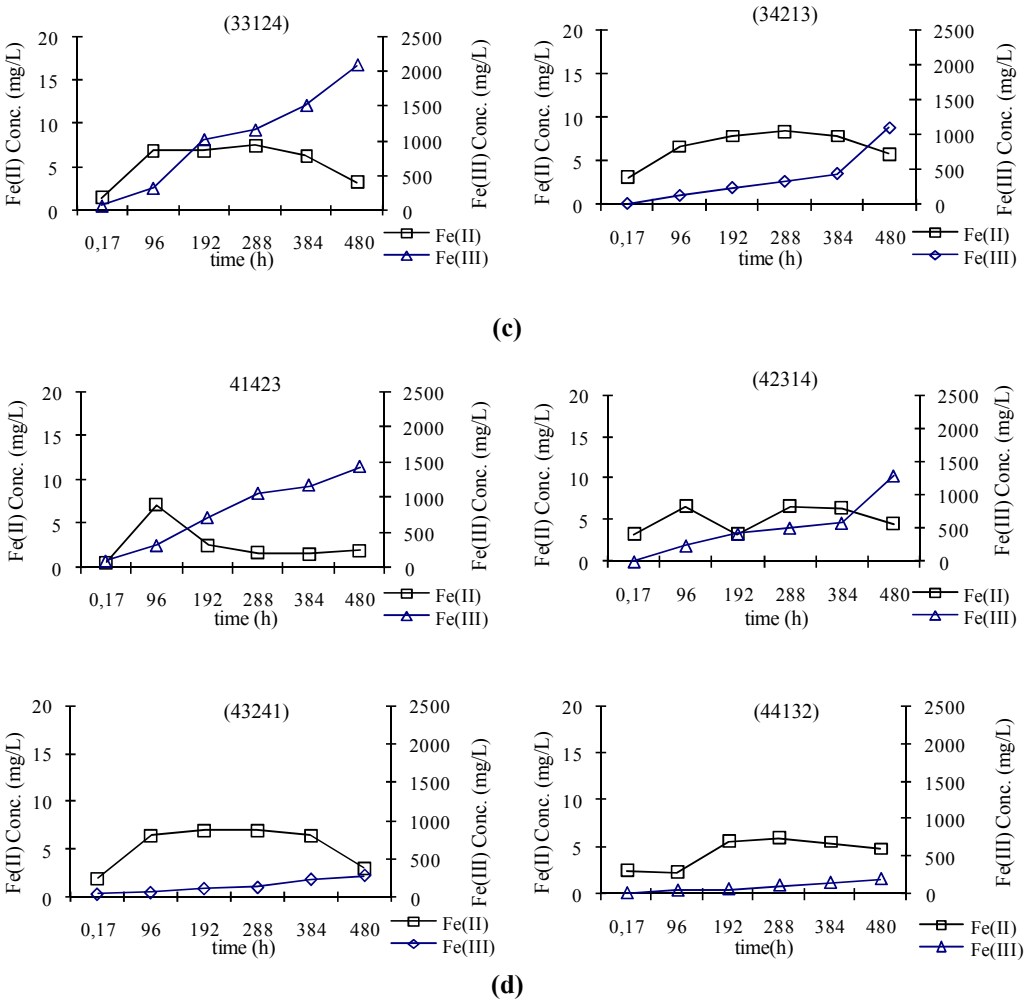


Fig. 3: Variation of the Fe(II) and Fe(III) concentrations (a;pH=1.50, b;pH=2.0, c;pH=2.25, d;pH=2.50).

All of the important bacteria in the biooxidation are acidophilic and exhibit their performance between 1.2 and 2.3 pH. According to (Oliver and Vanslykee, 1998), oxidation of iron and of ferrous ion involves the movement of hydrogen ions and electrons therefore; pH shows an important effect on metabolism [26]. Several authors agree that the optimal pH levels to avoid inhibition and precipitation are between 1.7 and 2.0 [36]. In considered previous studies, it was seen that the as pH raised; iron precipitation percent also raised but, when the pH was fall under 1.50, iron precipitation percent was also reduced but a lower bacterial activity was seen [10],. When pH of solution decreased, the bacteria had a greater effect on the rate of dissolution of pyrite. At the surface pH might rise by the bacterial consumption of H^+ in the oxidation of ferrous ions.

The lowest mean iron efficiency was measured at pH=1.50 in the all experiments here. The iron efficiencies were had similar amounts at pH=2.25 and 2.50. But the highest efficiency was obtained at pH=2.00. In this pH value, the total iron efficiency was determined as13.8% at first 192 h, and, it reached 44.6% at the end of 480 h. The rising of iron efficiency was same for each of four pH values at the end of 96th h, and, the differences between the iron efficiencies were seen after the 96th h. pH must be maximum 2.5 for the solubilization of the metals, especially iron oxidation. pH values between 2.0 and 2.5 were accepted to be optimum for bacterial oxidation of Fe(II) and sulfur.

It was observed that the pH increased until 96th h. This was due to the adaptation time of bacteria to the ore and bioleaching medium. After this adaptation time was completed, it was determined that the oxidation was begun and the pH changed

within the reasonable limits. The pH shortfalls were also seen in some experiments. When the experiments in which pH decreased were examined, it was seen that the bacteria could not found enough Fe(II) and Fe(III) concentrations or sulfur contents for completing the bioleaching process. Fe(III) concentrations showed a linear increase in all experiments. The effects on Fe efficiencies of the parameters in bioleaching optimization experiments were observed; lowest efficiency was obtained at pH=1.50 but maximum efficiency was obtained at pH=2.00. Besides, the jarosite formations were also affected from the pH increases (Fig. 4).

The iron oxidation rate increased with increase of pH up to 1.75 and thereafter it showed a downward trend[40]. When Fe(III) existed in the medium, this affected negatively iron oxidation rate. But, when Fe(II) existed in the medium, this was mixed affected to iron oxidation rate. Equally, firstly Fe(II) concentration was being rise then it was trend the reduced in the bioleaching experiments. In this way, the rise of Fe(III) concentrations were affected with the oxidation of the Fe(II) and it was shown that the higher oxidation rates were obtained at the optimum pH values. According to (Olubambi et al 2008), since redox potential depends on the Fe^{3+}/Fe^{2+} ratio, higher Fe^{2+} concentration within the system would affect the ferrous/ferric atio in the system. The initial low solution potential could therefore be an indication that the ferrous ions were not oxidized at the initial stage of bioleaching. This could have also resulted from the initial decrease in the bacteria cells within the system resulting in a corresponding decrease in the oxidation of ferrous ions.

The increase in the growth and mesophilic activity and interaction with minerals at low pH could be probably due to the fact that bacterial growth is usually higher at a very low pH value. It should be understood that H^+ is a very essential nutrient for the bacterial growth, because during the oxidation of ferrous ions to ferric ions, bacteria takes up H^+ ions from its external environment (Eqn.3) [38].

The bacterial interaction with metals at lower pH probably results from the effective competition by H^+ ions for negatively-charged sites at the cell surface [38].

According to (Fowler et al 1999), bacteria raise pH in the surface of mineral, namely, attached bacteria raise the leaching rate. When pH is higher than the 2.50, soluble iron hydrolyzes and then precipitates, and the jarosite formation is seen. Jarosite was the most abundant phase identified at pH of 2.50, but none or few was identified at pH 2.00 and 2.25. This situation also causes to decrease amount of Fe(III), oxidant of sulfur minerals and the Fe(II) [40]. Since the combined effects of bacteria-mineral interaction, hydrolysis of ferric iron, and sulphur oxidation lead to an increase in acidity, bioleaching at longer times resulted in reduced pH. According to (Olubambi et al 2008), the implication of this is that the initial pH at 1.8 could be reduced as indicated by the increase in solution potential, and the decrease in pH would presumably hinder the formation of jarosite.

*Optimum Parameters***:** In the result of evaluation of the data obtained in the bioleaching experiments, the optimum parameters for 192nd h and 288th h were pH; 2.00, the pulp density; 4% (w/v), inoculum volume; 4% (v/v), stirring rate; 120 rpm and particle size; -0.053 mm and for 384th h and 480th h were pH;2.00, the pulp density; 4% (w/v), inoculum volume; 5% (v/v), stirring speed; 200 rpm and particle size; -0.053 mm. The confirmation experiments were carried out with these optimum parameters and Fe extractions were investigated by these confirmation experiments. Fe extraction efficiencies and concentrations are given in the Table-9.

Table-9: Fe concentrations and efficiencies of the confirmation experiments (according to the optimum parameters for 192nd and 288th h, 384th h and 480th h)

Symbol	Time (h)	Fe(II) concentration (mg/L)	Fe(III) concentration (mg/L)	Fe efficiency (%)
Opt-192	192	2.63	1245.93	58.2
Opt-288	288	2.05	1669.74	76.6
Opt-384	384	2.04	1931.62	88.6
Opt-480	480	0.52	2135.57	97.9

Biooxidation cycles in the bioleaching experiments were carried out at the optimal temperature for bacteria. According to (Bosecker, 1997), the optimum temperature for ferrous iron and sulfide oxidation by *A.f.* was between 28-30 ºC. The temperature was eventuated at 23-28 ºC in the bioleaching experiments. The principal cause of carrying out of the experiments on this temperature interval was adaptation of the bacteria at this temperature or lower temperatures during the isolation procedures. The control experiments were also carried out in bacteria-free medium with only distillated water instead of 9K medium (Table-10).

Table-10: Mean Fe concentrations and efficiencies of the control experiments.

EXPERIMENT NAME	INOCULUM VOLUME (mL)	PULP DENSITY (g)	TOTAL SOLUTION VOLUME (mL)	SOLUTION pH	STIRRING CONDITION (rpm)	PARTICLE SIZE (mm)
Distillated water	0	20 (%4)	500	2.00	120	-0.053

SAMPLE NO	TIME (h)	Mean Fe concentrations in solution after bioleaching (mg/L)			Total Fe in the ore (mg/L)	MEAN TOTAL Fe EFFICIENCY (%)
		Fe(II)	Fe(III)	TOTAL Fe		
1	0.17	0	0	0	2099.0	0
2	96	10.81	0	10.81	2099.0	0.5
3	192	13.00	0	13.00	2099.0	0.6
4	288	15.57	0	15.57	2099.0	0.7
5	384	13.84	346.43	360.27	2099.0	17.2
6	480	4.54	383.95	388.50	2099.0	18.5

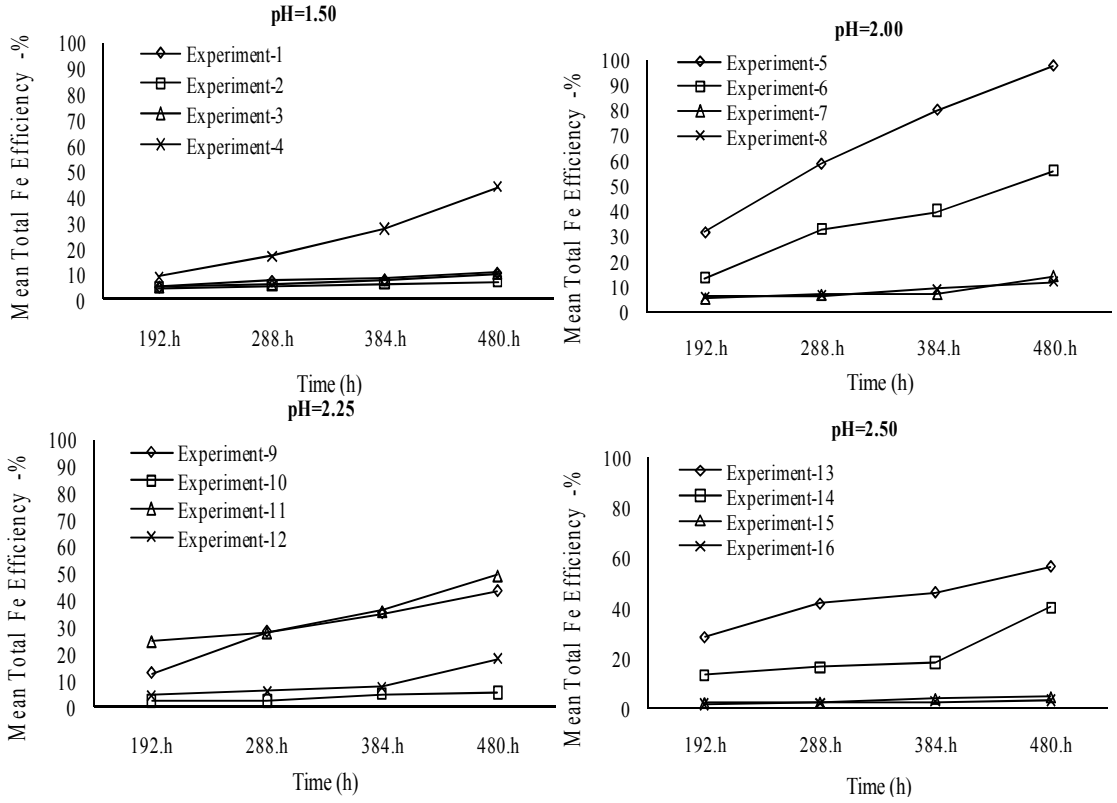


Fig. 4: Effects of pH on Fe efficiency (according to the Table-6 and 8).

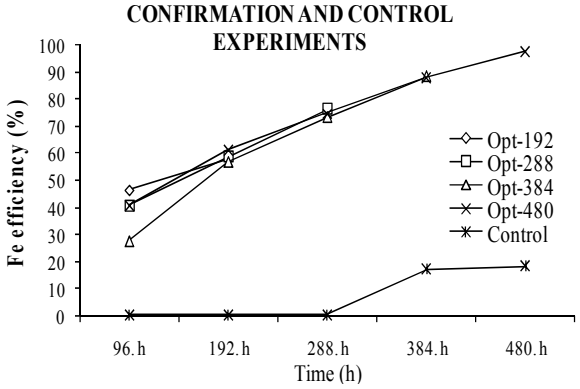


Fig. 5: Fe efficiencies of the confirmation and control experiments for 192nd, 288th, 384th and 480th h.

Results of control experiments carried out with only distillated water were compared with results of confirmation experiments obtained at the end of 480 h (Fig. 5). Although in the control experiments, Fe oxidation began at the end of the 288th h,it began before 96th h in the confirmation experiments. While almost 100% iron extractions was obtained in the confirmation experiments at the end of 480th h, almost 5 times low efficiencies was obtained in control experiments.

Conclusions

The optimum parameters for 192nd h and 288th h were pH;2.00, the pulp density; 4% (w/v), inoculum volume; 4% (v/v), stirring rate; 120 rpm and particle size; -0.053 mm and for 384th h and 480th h were pH;2.00, the pulp density; 4% (w/v), inoculum volume; 5% (v/v), stirring speed; 200 rpm and particle size; -0.053 mm in the bioleaching optimization experiments.

The confirmation experiments were carried out with these optimum parameters and Fe extractions were investigated by these confirmation experiments. Fe extraction efficiencies and concentrations were 58.2% at 192nd h, 76.6% at 288th h, 88.6% at 384th h and 97.9% at 480th h. Fe extraction efficiencies were also 0.6% at 192nd h, 0.7% at 288th h, 17.2% at 384th h and 18.5% at 480th h in the control experiments.

At the end of the experiments, it was observed that the bioleaching has a very fast oxidation rate according to the control experiments which were carried out with only distillated water.

Abbreviations

AMD: Acidic Mine Drainage
MTA: General Directorate of Mineral Research & Exploration
A.f.: Acidithiobacillus ferrooxidans
EDTA: Ethylene Diamine Tetraacetic Acid

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