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Bioleaching of high grade Zn–Pb bearing ore by mixed moderate thermophilic microorganisms



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ABSTRACT

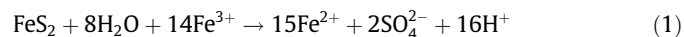
In this research, the bioleaching mechanism of zinc and lead from high-grade Zn–Pb ore has been investigated. It is done by using mixed culture of iron and sulfur oxidizing moderate thermophilic bacteria at 45 °C. Pulp density, initial pH and ferrous concentration were studied as influential parameters in bioleaching experiments. The optimum conditions were achieved at pulp density = 50 (g/L), initial pH = 1 and FeSO₄·7H₂O concentration = 75 (g/L) with 98.5% zinc recovery after 25 days treatment. Generally, an increase in ferrous concentration caused an improve zinc recovery, and an increase in initial pH and pulp density caused reduction in zinc recovery. However, in the test with optimum condition the lead dissolution was just 0.027% due to the lower Pb solubility. Furthermore, cadmium dissolution was 98% under optimum condition and results showed the cadmium dissolution was in direct proportion with zinc dissolution. Finally, 7.82% of arsenic and 8.52% of antimony dissolved during zinc bioleaching after 25 days treatment, both under above mentioned optimum condition.

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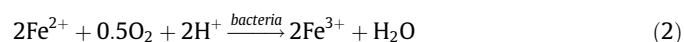
1. Introduction

Biohydrometallurgical methods for processing and remediation consume less energy and are eco-friendly and emit no harmful gases in the environment [1–6]. These processes are based on the ability of specific bacteria and fungi to dissolve refractory minerals in form of extractable elements which can be recovered. This ability has attracted scientific and industrial attention during recent decades for its advantages, such as low environmental impacts, low energy requirement, simple processes, and being applicable for all mining production which include concentrate, high grade ore and low grade mine tailings and residues [7–10]. Thanks to these advantages and less negative effects, many researches has been conducted on application of biotechnology on extracting metals from low grade Zn and Pb ores and concentrates [11–16]. Previous studies have proposed two mechanisms to explain the sulfide minerals bio-dissolution which are direct and indirect mechanisms. By direct mechanism, bacteria contact to the mineral surface and oxidize it through enzymatic reactions. However indirect mechanism suggests that bacteria regenerate the oxidizing agent of the mineral by means of the oxidation of ferrous ions to

ferric ions via thiosulfate or polysulfide, depending on the type of minerals [17–21]. In fact with these mechanisms, bacteria play the role of catalyst and increase dissolution rate of sulfide minerals. Part of this study investigates the possibility of replacing FeSO₄·7H₂O with pyrite. The previous studies have shown that ferric ions which are produced during iron-sulfide oxidizing bacteria activity have a great influence on sulfide minerals dissolution [22–24]. Thus, almost all culture media that are used in bioleaching process include a source of iron, which mostly is FeSO₄·7H₂O. However, adding high amounts of ferrous sulfate to industrial bioleaching systems is commercially and technically impossible. Therefore the possibility of replacement of sulfate iron with pyrite was studied. Fe (II) is produced from pyrite by following reaction [25]:



Ferrous ions are re-oxidized to ferric ions in presence of bacteria by below reaction:

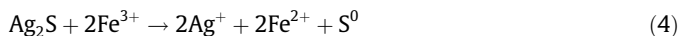


Prior investigations also have suggested catalyst effect of silver ions on bioleaching process [26–29]. Silver ions participate in dissolution process in following reactions [30]:



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In which MeS signifies sulfide minerals in the form of $\text{Me}^{2+}\text{S}^{2-}$ (e.g., PbS or ZnS). Ferrous ions are re-oxidized to ferric ions in the presence of bacteria and complete the dissolution cycle. To study the effects of Ag^+ on bioleaching of this particular ore, AgNO_3 is added to one of shaking flask test.

Almost in all mineral processing methods one of the costly steps is size reduction by passing through crushers and mills circuit. Using ore with particles size less than 2000 micro-meter (10 MESH) removes the necessity of using mills circuit which in turn significantly save the energy cost. This study was designed to study the kinetically behavior of bio-dissolution of high-grade large size particles of Zn–Pb ore. In addition, the arsenic, cadmium and antimony dissolution during Zn–Pb bioleaching were monitored. Moreover, the effects of initial pH, ferrous concentration and pulp density were study to achieve the highest dissolution rate.

2. Materials and methods

2.1. Ore sample

In this study, a high-grade Zn–Pb ore sample was taken from Anguran Zinc & Lead Mine (Zanjan, Iran) which was used in all chemical and bacterial experiments. Using roll crusher (Danesh Faravaran, Iran), the ore sample was crushed and screened to 80% smaller than 2 mm with ASTM sieves in a closed circuit. Chemical analysis showed that the main components were zinc and lead. The total Zn and Pb contents were 40.71% and 12.4%; respectively. Furthermore, the ore sample included 1.1% iron, 3621 mg/kg arsenic, 2340 mg/kg cadmium and 115 mg/kg antimony. The optical mineralogical study and X-ray diffraction (XRD) analysis (Inel EQUINOX3000, USA) showed that the main minerals were sulfide minerals consisting sphalerite (ZnS) and galena (PbS). In addition, some small quantities of carbonate minerals were present in the sample which included smithsonite $\text{Zn}(\text{CO}_3)$ and cerussite $\text{Pb}(\text{CO}_3)$. Also calcite $\text{Ca}(\text{CO}_3)$ and quartz (SiO_2) were identified as gangue minerals.

2.2. Microorganisms

The original microorganisms were collected from water and soil samples which were gathered adjacent to Anguran Mine and they were enriched in 9 k media. This mineral salts medium contained $\text{Ca}(\text{NO}_3)_2$ 0.01 (g/L), KCl 0.1 (g/L), K_2HPO_4 0.5 (g/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 (g/L), $(\text{NH}_4)_2\text{SO}_4$ 3.0 (g/L), and $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ 44.22 (g/L) [31]. In addition, 1 g of elemental S was added to each flask to improve the growth's speed of sulfur oxidizing bacteria [32]. The cultures were grown in 250 ml shake flasks with 120 rpm, each containing 100 ml medium 45 °C for isolating moderately thermophilic acidophilic chemolithotrophic consortia (TACC). Growth was monitored by the visual observations using optical microscope (KRUSS, Germany). The average microbial concentration that was used for bioleaching treatments was enumerated 4×10^7 cells/ml by counting under optical microscope using Neubauer counting chamber.

2.3. Bioleaching experiments

Shaking flasks studies were conducted in 250 ml Erlenmeyer flasks containing 90 ml of 9 K medium and 10 ml of enriched bacteria. Three effective parameters, namely initial pH, Fe(II) concentration and pulp density were selected for study. Design-Expert 7 (DX7) software was used for experiments designing to reduce the tests numbers and optimizing them. Response surface method (RSM) was selected to design the tests. To study the above mentioned parameters, three categorical factors with three different

levels were selected. These included initial pH at 1, 1.5 and 2, initial ferrous sulfate concentration at 25 (g/L), 50 (g/L) and 75(g/L) and pulp density at 50 (g/L), 100 (g/L) and 150 (g/L). To study the chemical dissolution and compare it with bacterial dissolution, some tests were repeated without any bacteria, as control test. To disinfect the environment, 3 ml formaldehyde 37% was added to these flasks. In addition 0.1 g of AgNO_3 was added to test 13 for investigating the effect of silver ions on efficiency of zinc and lead bioleaching. In test 14, the $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was replaced by pyrite to study the possibility of replacing Fe^{2+} with pyrite mineral. Different conditions of experiments are listed in Table 1. The pH was adjusted with diluted sulfuric acid and Fe(II) was added as $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ (Merck, Germany). The solution samples were withdrawn at the same intervals and the dissolved zinc, lead, iron and cadmium were determined by atomic absorption spectroscopy (AAS) (Varian AA 240Z) and arsenic and antimony were analyzed by atomic fluorescence spectroscopy (AFS) (AF-640A, China). All flasks were shaken in a shaker incubator (Shin Saeng SKIR-601, Korea) at 45 °C and 120 rpm for 25 days. Deionized water was added to the flasks to compensate for evaporation losses.

3. Results and discussion

3.1. pH and oxidation–reduction potentials (ORP)

Generally, the bacteria that were used in bioleaching process obtain energy by oxidation of either ferrous ions to ferric or sulfur compounds to sulfuric acid [33–35]. In most acidic bioleaching systems, the oxidation–reduction potential (ORP) predominantly show the $\text{Fe}^{3+}/\text{Fe}^{2+}$ ratio and the activity of iron-oxidizing bacteria [14,36]. Therefore, monitoring the pH and redox potential can show the bacterial activity and oxidation rate. Fig. 1 shows the pH fluctuations during 25 days treatment for 22 different experiments. The pHs increased during a short period, thereafter decreased for about 7 days which was due to the start of bacterial activities and then became constant. As mentioned before, the ore contained carbonate minerals as minor. The pH increased due to dissolving these carbonate minerals as well as using H^+ in reaction with CO_3^{2-} . In test 14 which was studying the replacement of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ by pyrite, the pH was lower than tests 12 and 13. Pyrite is oxidized during following biochemical reactions:

Table 1
Conditions of the moderate thermophilic bioleaching experiments.

No	pH	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (g/L)	Pulp density (g/L)	Type of test
1	1	25	50	Bioleaching
2	1	25	100	Bioleaching
3	1	25	100	Control
4	1	25	150	Bioleaching
5	1	50	50	Bioleaching
6	1	50	50	Control
7	1	75	50	Bioleaching
8	1	75	50	Control
9	1	75	150	Bioleaching
10	1.5	25	50	Bioleaching
11	1.5	25	150	Bioleaching
12	1.5	50	100	Bioleaching
13	1.5	50	100	AgNO_3
14	1.5	–	100	Pyrite
15	1.5	75	50	Bioleaching
16	2	25	50	Bioleaching
17	2	25	50	Control
18	2	25	150	Bioleaching
19	2	50	100	Bioleaching
20	2	75	50	Bioleaching
21	2	75	150	Bioleaching
22	2	75	150	Control

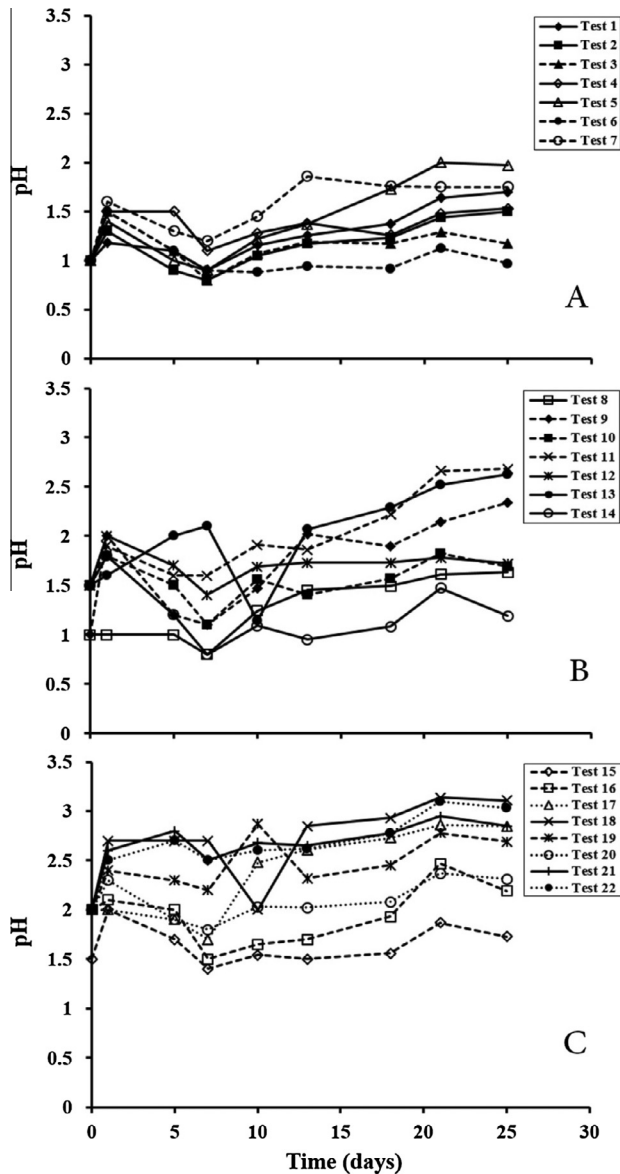
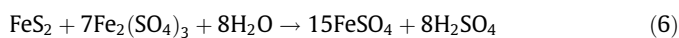
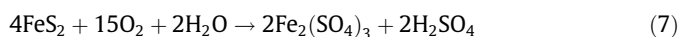


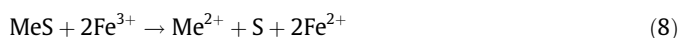
Fig. 1. The pH fluctuation during 25 days of bioleaching treatment.



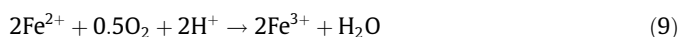
The overall summary of pyrite oxidation is as follows:



On the other hand, the microbial leaching of galena or sphalerite often proceeds through an indirect mechanism in following relations [37]:



where MeS signifies sulfide minerals in the form of $\text{Me}^{2+}\text{S}^{2-}$ (e.g., PbS or ZnS)



The above reactions show, during the pyrite oxidation 2(mol) H_2SO_4 is produced while during the oxidation of sphalerite or galena

1(mol) H_2SO_4 is produced and this is the main reason for lower pH in test 14 in compare to tests 12 and 13.

Fig. 2 shows oxidation–reduction potential (mV, Ag0/AgCl reference) changes in different flasks during experiments. Generally, experiments did not have very sharp ORP changes during treatments. For example, redox potential for test 7 which contained the highest zinc dissolution, changed between 319 and 352 mV. Also, the OPR for test 12 that contained 50 (g/L) $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ increased from 373 (mV) to 518 (mV) and then became almost constant in range of 500 (mV). This shows the high rate of oxidation of ferrous to ferric ions by moderate thermophilic bacteria in this test. On the other hand, test 13 (contained silver ions) and test 14 (contained pyrite instead of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) had almost constant ORP in range of 250–270 (mV) and 240–250 (mV); respectively. Counting cells under optical microscope showed very low bacterial concentration for test 13 which had caused the smooth changes in

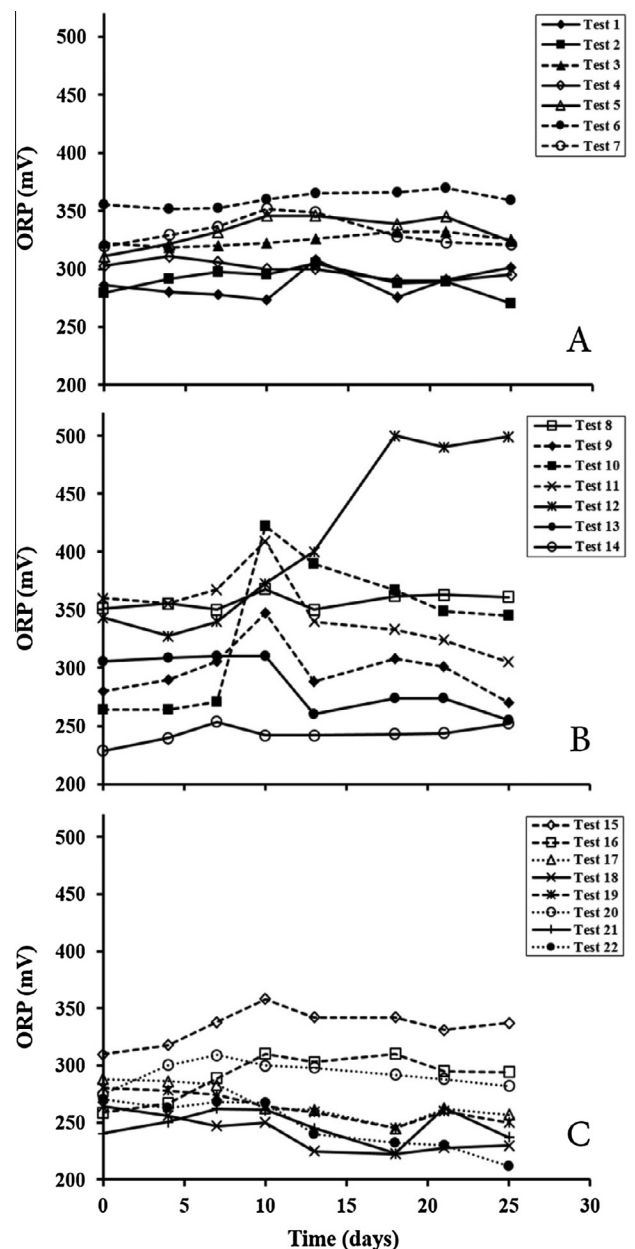


Fig. 2. The oxidation–reduction potential (mV) changes during 25 days of treatment.

redox potential for this test. Test 14 had smooth ORP changes due to absence of free ferrous ions in solution.

3.2. Zinc dissolution

3.2.1. Optimization of parameters

The zinc extractions at different tests during 25 days are shown in Fig. 3. The highest zinc recovery (98.5%) was obtained from the test with pulp density 50 (g/L), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ concentration 75 (g/L), and $\text{pH} = 1$ (test 7). The final zinc concentration was 20.25 (g/L) in this test. The zinc recovery in control test with these conditions (test 8) was 67.57% with the zinc concentration 13.75 (g/L). Reducing the $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ to 5 g (test 5) caused reduction in zinc recovery to 81.18%. The test with lowest Zn recovery (15.31%) had 150 (g/L) ore, 25 (g/L) $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ with initial $\text{pH} = 2$ which contained 9.35 (g/L) zinc.

Statistical analysis is necessary, not only to optimize the mentioned parameters but also to study the interactions between

parameters. Design Expert 7 (DX7) software and response surface methodology (RSM) is applied to analyze the data statistically. The final zinc recovery was selected as response. According to the software suggestion, a linear model could be fitted to data. Table 2 that shows the analysis of variance (ANOVA) for zinc recovery indicates three mentioned parameters have significant effect on zinc recovery. There is an interaction between pH and pulp density, however the interactions between other parameters have been removed from mathematical model due to high P -Value for them. Eq. (11) shows a mathematical model that can related the effective parameters and their interactions to zinc recovery, where Fe signifies the $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ concentration and PD signifies the pulp density both in g/L. This model is highly significant with P -Value less than 0.0001 and *Adequate Precision* 15.231. This means that the proportion of signal effects to noise effect for this model is more than 15.

$$R_{\text{Zn}} = +161.47336 - (65.64886 \times \text{pH}) + (0.2214 \times \text{Fe}) - (0.8431 \times \text{PD}) + (0.34964 \times \text{pH} \times \text{PD}) \quad (11)$$

As Eq. (11) shows, increasing in the ferrous concentration has positive effect on zinc bio-dissolution while increasing in pH and pulp density have negative effect. According to reaction 2, increasing the Fe^{2+} causes producing more Fe^{3+} by bacteria that accelerate the sulfide minerals dissolution. On the other hand, by increasing the pulp density the proportion of solvent to free surface will decrease that will cause reducing the minerals dissolution.

The optimization of parameters also carried out. The target is maximizing the zinc dissolution while $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ concentration becomes minimize and pulp density becomes maximize. This strategy could reduce the final production cost. According to DX7 software calculation, the highest zinc bio-dissolution could be achieved when pulp density is 50.42 g/L, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ concentration set on 73.45 g/L and $\text{pH} = 1.00$. Deviation from these conditions will cause reducing the final zinc recovery.

3.2.2. Effects of silver and pyrite

Test 13 was performed to study the effects of silver ions on bioleaching experiment. The final recovery for this test was 38.82% and for test 12 (the test similar to test 13 but without silver ions) was 46.92%. As Fig. 3B shows, in first 12 days the zinc dissolution was higher for test 13 in compare with test 12. Also, after 12 days the zinc dissolution for test 12 started to increase and became higher than test 13. Counting bacteria under optical microscope using Neubauer counting chamber showed that bacterial counts in test with silver ions were lower than test 12 due to toxicity of silver for bacteria. The bacterial count started to increase in flask 12 after about 10 days lag period and along which, as Fig. 3B shows, the zinc dissolution started to increase too. On the other hand, the slop of Zn extraction-Time diagram for test 13 was almost constant. These points show that although the silver ions increased the chemical leaching rate, they decreased the bacterial leaching rate.

Test 14 shows the effects of replacing $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ with pyrite. The final zinc recovery for this test was 20.88%. With these properties bacteria oxidized pyrite as iron source and produced ferric ions that in turn, were used in zinc leaching process. This caused lower zinc dissolution in compare to test 12. In fact, adding pyrite instead of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ causes a delay period in zinc dissolution and increases the leaching time.

3.3. Lead dissolution

Generally, the Pb dissolution recoveries were very low and they were under 0.3%. The highest recovery was just 0.271% which were obtained from flask with 50 (g/L) ore, 75 (g/L) $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and

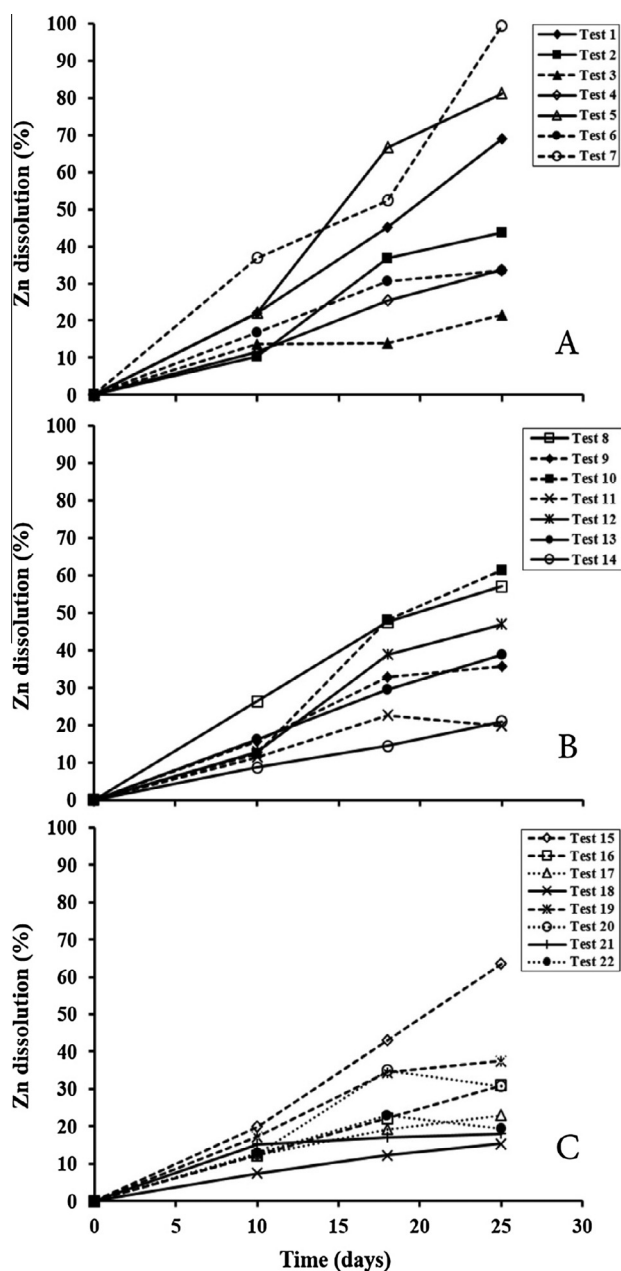


Fig. 3. Zn recovery in different condition after 25 days of treatment.

Table 2
Analysis of variance for zinc recovery.

Source	Sum of squares	Degree of freedom	Mean square	F-Value	P-Value
Model	7539.54	4	1884.89	28.81	<0.0001
pH	2507.9	1	2507.9	38.34	0.0002
FeSO ₄ ·7H ₂ O	319.34	1	319.34	4.88	0.0545
Pulp density	2626.44	1	2626.44	40.15	0.0001
pH × Pulp density	671.08	1	671.08	10.26	0.0108
Residual	588.76	9	65.42		
Cor. Total	8128.3	13			

pH = 1 (test 7) after 10 days treatment. The lead dissolution for test 13 (the test which included silver) was less than test 12. For test 14 (test with pyrite), the dissolved lead decrease after a period of dissolving and then started to increase again. The second dissolving period started when pyrite was oxidized and produced Fe³⁺ by bacteria and these ferric ions were in turn used in other sulfide minerals oxidation. Also, the final lead dissolution for test 14 was higher than other 2 tests. The results show, although the pregnant leaching solution included a high concentration of zinc, it contained very low concentration of lead. Thus, during the zinc extraction stage (by electro-winning, sedimentation or other methods), it would become possible to produce pure zinc bullion. In addition, the solid residual did not contain zinc and it could be used as lead concentrate and can be processed in other methods of purification such as roasting and pressure leaching. Lead dissolutions in different conditions are demonstrated in Fig. 4.

3.4. Other metals dissolution

The Fe₂SO₄·7H₂O that was added to flasks and the iron bearing minerals were two major iron resources. Fig. 5 shows changes in dissolved iron in 22 tests during 25 days treatment. According to this figure, the iron concentrations in tests with initial pH = 1 (test 1–9) were almost constant for first 10 days, and after that they started to increase due to bio-dissolution of minerals that contain iron. The Fe Concentrations in control tests with pH = 1 were constant or decrease during 25 days processing. Although, iron concentrations for tests with initial pH = 1.5 (tests 10, 11, 12) were almost fixed during first 10 days, they decrease after this period. For test with pH = 2 however there were two different trend. There was a slight fluctuation in iron concentrations for tests with low Fe₂SO₄·7H₂O concentration. On the other hand, the Fe declined in flasks that contained 75 g/L Fe₂SO₄·7H₂O after 10 days. This decreasing trend in dissolved iron can be caused due to jarosite (ferric hydroxide) precipitation. Precipitation of jarosite is typically described by the following reaction [25] where potassium may be replaced by Na⁺:



The Fe²⁺ that was added to flasks was oxidized to Fe³⁺ by bacteria and some part of these ferric ions precipitated as jarosite by above reaction. The previous study showed that ferric hydrolyzes is sensitive to the pH and critical pH is calculated according to below formula [38]:



$$K = \frac{(Fe^{3+})(OH^-)^3}{Fe(OH)_3} = 1.58 \times 10^{-39} \quad (14)$$

$$(OH^-)^3 = \frac{1.58 \times 10^{-39}}{Fe^{3+}} \quad (15)$$

Eq. (14) shows if solution contains 0.01 (mol) ferric ions, the pH of precipitation will be 1.7. Therefore, jarosite precipitation rate can be adjusted by controlling pH. In addition, the iron precipitation could

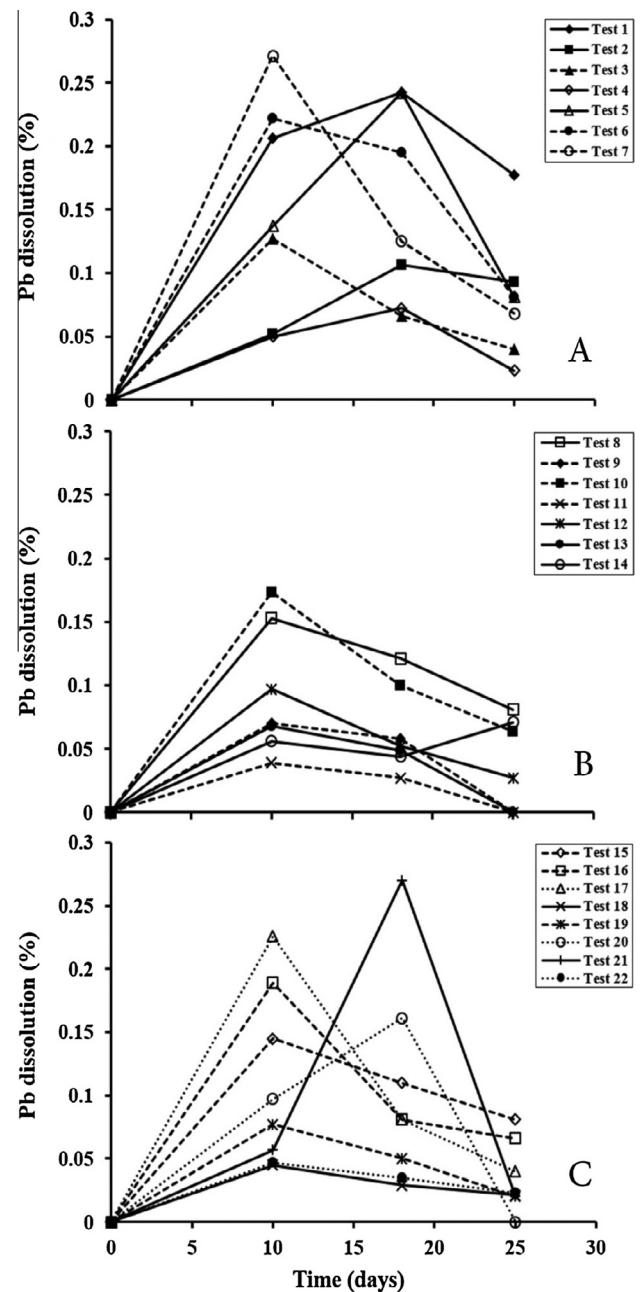


Fig. 4. Pb dissolution in different conditions after 25 days of treatment.

be controlled by transferring of the ore to the new media. Generally, jarosite precipitation during bioleaching is a negative phenomenon due to removal of ferric ions from solution, and this causes reduction of the leaching rate. In addition, jarosite makes a thin film on minerals surfaces and stop the leaching process. So it is necessary

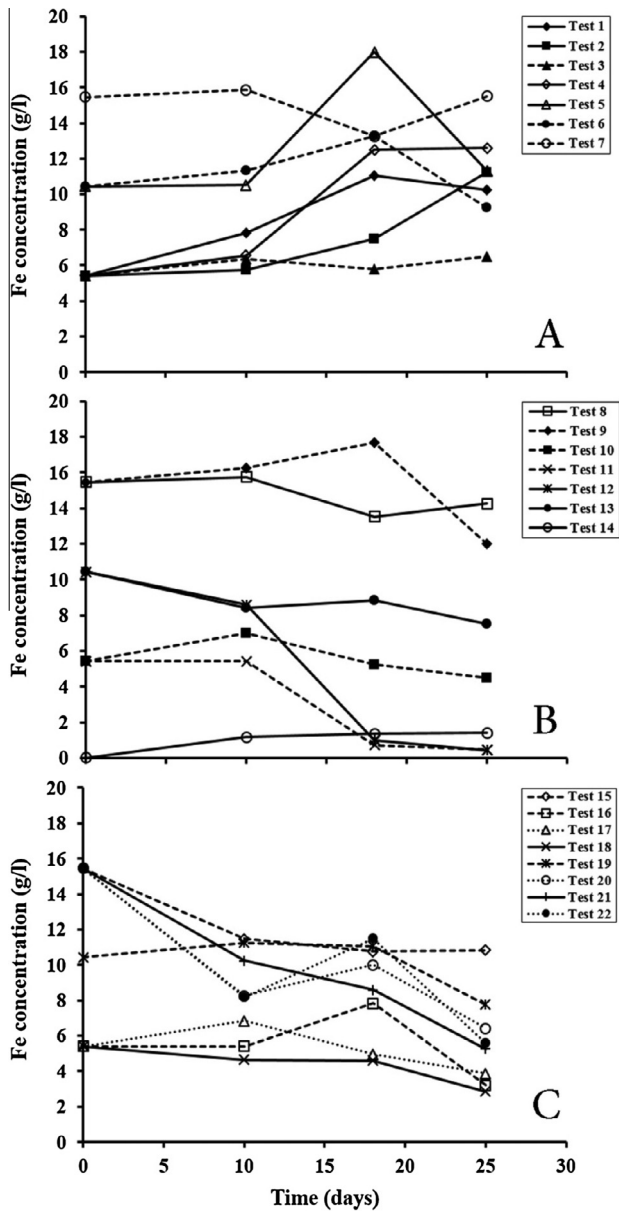


Fig. 5. Dissolved Fe in different tests condition during 25 days treatment.

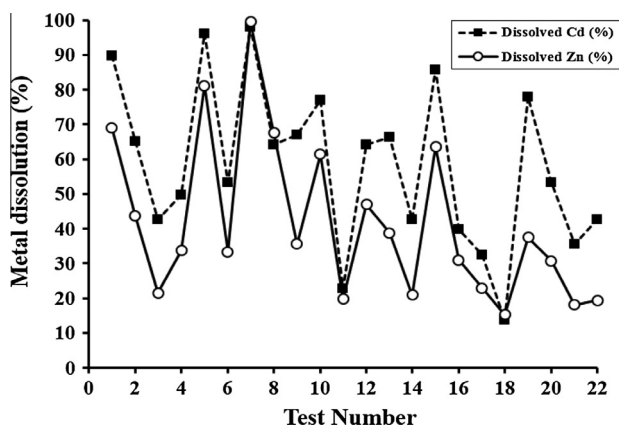


Fig. 6. Final Zn & Cd recoveries in different tests.

to monitor the soluble iron during bioleaching experiments. For the test with pyrite as energy source (test 14), the total dissolved iron increased in linear slop which shows the pyrite oxidization and production of iron ions. Reduction in the dissolved iron for test with silver ions (test 13) was less than test without silver ions (test 12). The ferric ions were less in this test due to lower bacterial count in test 13, thus the jarosite precipitation was less too and the total dissolved iron was more. This confirms that metals dissolution for test 13 was controlled by chemical process rather than bacterial process.

Acid mine drainages (AMD) is one of the most important problems in mining industries. This metal dissolution of tailing dump can cause lots of environmental problems [39–45]. Removal of the heavy metals can reduce the toxicity of these drainages. On the other hand, the high concentrations of these metals in pregnant leaching solution reduce the quality of pregnant leach solution and cause some problems in downstream processes. These dissolved metals can be settled down in the next steps. Therefore,

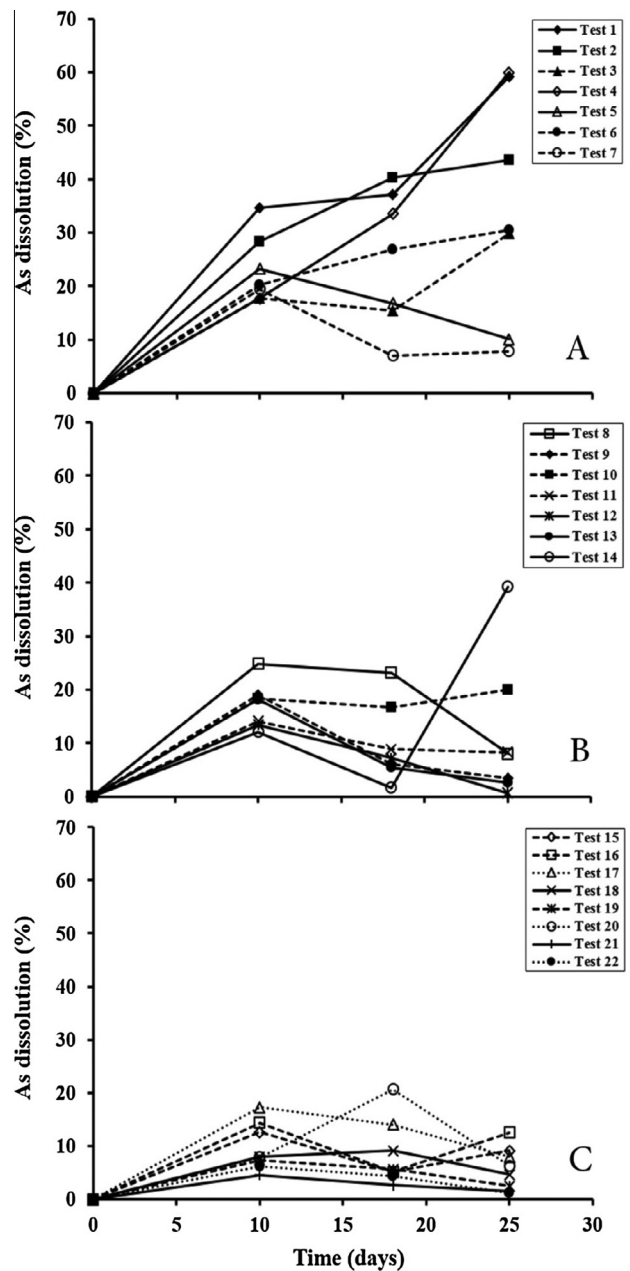


Fig. 7. Dissolved As in different tests during 25 days of treatment.

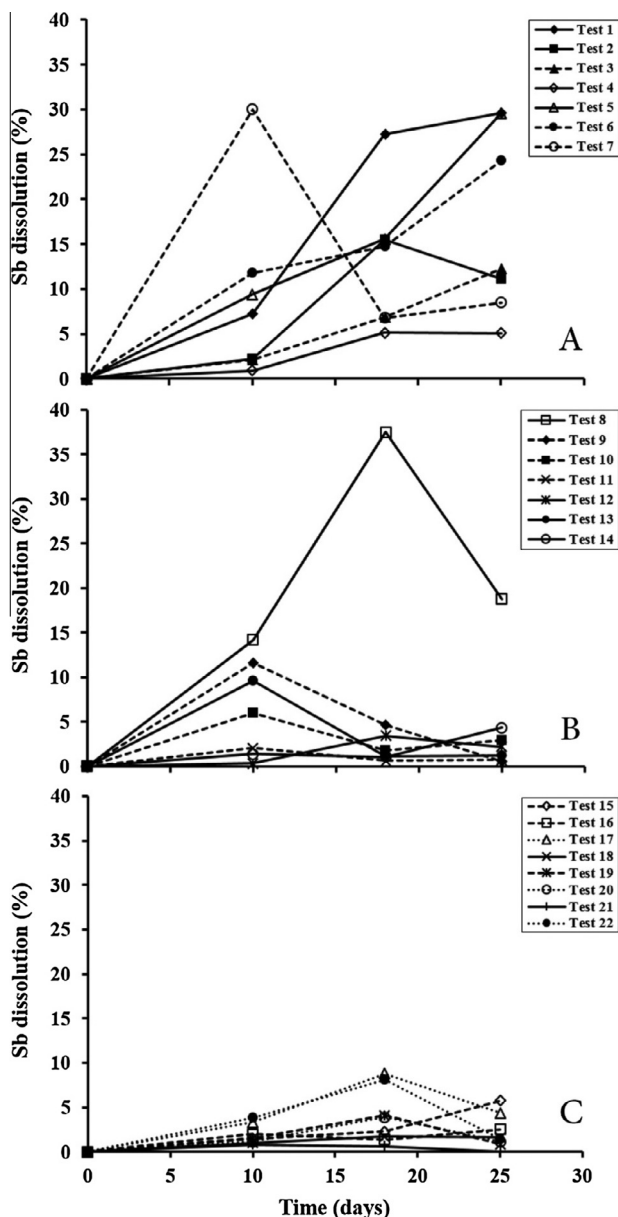


Fig. 8. Dissolved Sb in different condition during 25 days treatment.

monitoring these heavy metals in soil and water is essential for decreasing environmental impacts and increasing pregnant leach solution quality. The arsenic and antimony dissolutions were analyzed by atomic absorption spectroscopy during treatment and also cadmium, silver and copper were analyzed in final pregnant leach solution. The atomic absorption spectroscopy analysis of final

pregnant leaching solutions showed that the concentration of copper and silver were almost zero in all flasks.

On the other hand, the highest final cadmium dissolution (98%) belonged to test with pulp density 50 (g/L), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ concentration 75 (g/L) and pH = 1 (test 7). The cadmium dissolution in control test (test 8) was 64.1%. Test 7 had the highest zinc recovery too. The lowest cadmium recovery was for test 18 with pulp density 150 (g/L), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 75 (g/L) and initial pH = 2. This test, also, had the lowest zinc recovery. Fig. 6 shows Zn and Cd final recoveries; simultaneously. As this figure shows, cadmium and zinc dissolutions are directly proportionate.

Fig. 7 shows arsenic fluctuations in different flasks during experiments. The highest final dissolved arsenic was 59.94 (%) which was obtained from the test 1 with pH = 1, pulp density = 50 (g/L) and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ = 25 (g/L). This test included 0.033 (g/L) dissolved arsenic. In addition, the test with optimum conditions (test 7) contains 0.042 (g/L) arsenic after 10 days of processing, 0.030 (g/L) after 18 days and 0.018 (g/L) arsenic after 25 days of processing. It shows, although the dissolved zinc increased during 25 days bioleaching, the dissolved arsenic decreased after 10 days treatment. This decrease in dissolved arsenic causes an increase in pregnant leach solution quality for solvent extraction/electro-winning (SX/EW) process.

Fig. 8 depicts antimony recoveries during 25 days experiment. After 10 days processing, test 7 (test with optimum condition for zinc recovery) had the highest antimony dissolution with 1.725 (mg/L) and 30% Sb recovery. Also, the Sb started to decrease after 10 days and became 0.39 (mg/L) with 6.78% antimony recovery after 18 days. Therefore, after this period pregnant leach solution quality increased due to decrease of dissolved antimony.

The slope of Sb dissolution for test 13 (test with silver ion) was very sharper in compare with test 12 or 14 during first 10 days. However, the dissolved antimony precipitated with a sharp slop after the 10th day. In test 14 (which contained pyrite), the dissolved Sb quantity was almost fixed during first 18 days, but it started to increase with a sharp slop after 18th day. On the other hand, in the test 12 which contained $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ instead of pyrite, the dissolved antimony increased during initial 18 days and decreased thereafter. These fluctuations reflect a delay period in bioleaching process in absence of free ferrous iron. As Fig. 8 shows, antimony dissolution is very sensitive to pH and an increase of pH to 2 causes a decline in the Sb recovery.

Similar to the zinc dissolution, response surface methodology (RSM) is used to determine the effective parameters and their interactions in lead, cadmium, arsenic and antimony bioleaching. Table 3 summarizes these effects for each element and shows mathematical models that control the bio-dissolution of them. According to calculations, the linear model is best fitted to all elements recoveries. However, the recoveries for lead transformed to square root format, arsenic recoveries transformed to base logarithm format, and antimony recoveries transformed to natural logarithm (Ln), according to software suggestion, to increase the models significance. Mathematical models for all elements are significant, as their *P*-Val-

Table 3

Effective parameters, their interaction and mathematical models for bio-dissolution of lead, cadmium, arsenic and antimony.

	Lead (Pb)	Cadmium (Cd)	Arsenic (As)	Antimony (Sb)
Response	$\sqrt{R_{pb}}$	R_{cd}	$\log(R_{as})$	$\ln(R_{sb})$
Constant factor	+0.89982	+127.15256	+3.35536	+5.44049
A-pH	-0.32091	-32.99349	-1.23407	-1.48001
B- $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	-1.90199×10^{-3}	+0.35675	-0.031691	-0.020536
C-pulp density	-5.35563×10^{-3}	-0.34969	0	-0.015295
A × B	0	0	0.013329	0
A × C	2.51133×10^{-3}	0	0	0
B × C	0	0	0	0
Model <i>P</i> -Value	0.006	0.0014	0.0015	0.0098

ues show. The numbers in the table are coefficients of parameters or interactions in mathematical models. The parameter or interaction has no effect on bio-dissolution, when coefficient is 0. The positive coefficient shows the positive effect on element bioleaching, and vice versa.

4. Conclusions

The zinc minerals were amenable to up to 98.5% dissolving during bioleaching process in 45 °C. Shaking flasks studies showed that the optimum conditions for achieving highest zinc dissolution were: pulp density = 50 (g/L), initial pH = 1 and FeSO₄·7H₂O concentration = 75 (g/L). An increase in pH and pulp density caused reduction in the zinc recovery, and an increase in Fe (II) concentration increased the Zn dissolution. The lead recovery in optimum condition test was just 0.271% and almost all Pb content remained in solid part. These bioleaching residuals could be used for producing Pb concentrate with a supplementary mineral processing method such as gravity separation. The results showed adding silver ions to bioleaching tests improved the chemical leaching speed kinetically; however it decreased the bacterial leaching rate due to reduction of bacterial counts because of toxic effect of Ag⁺ on bacteria which are not adopted properly. Moreover, replacing FeSO₄·7H₂O with pyrite decreased the final Zn dissolution which is caused by a lag phase in zinc bioleaching. There was a decrease of iron content in some tests which showed the Fe is precipitated probably in the form of jarosite. Comparing the two zinc and cadmium dissolution graphs showed a direct relation between them. 96.15% of cadmium was leached under optimum condition. More than 19% of arsenic dissolved in above mentioned optimum condition test during first 10 days, but the As precipitated and then, the final arsenic dissolution became 7.82%. Finally, the antimony dissolution showed a great sensitivity to pH and an increase in acidity to 2 caused a decline in Sb dissolution.

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References

- [1] A. Akcil, Potential bioleaching developments towards commercial reality: Turkish metal mining's future, *Miner. Eng.* 17 (2004) 477–480.
- [2] F. Anjum, H.N. Bhatti, M. Asgher, M. Shahid, Leaching of metals from black shale using organic acids produced by *Aspergillus niger*, *J. Appl. Clay Sci.* 47 (2010) 356–361.
- [3] C.L. Brierley, Biohydrometallurgical prospects, *Hydrometallurgy* 104 (2010) 324–328.
- [4] S.Y. Chen, P.L. Lin, Optimization of operating parameters for the metal bioleaching process of contaminated soil, *Sep. Purif. Technol.* 71 (2010) 178–185.
- [5] D.E. Rawlings, Microbially assisted dissolution of minerals and its use in the mining industry, *Pure Appl. Chem.* 76 (2004) 847–859.
- [6] F. Amiri, S.M. Mousavi, S. Yaghmaei, Enhancement of bioleaching of a spent Ni/Mo hydro-processing catalyst by *Penicillium simplicissimum*, *Sep. Purif. Technol.* 80 (2011) 566–576.
- [7] A. Akcil, H. Deveci, in: S. Jain, A. Khan, M.K. Rai (Eds.), *Mineral Biotechnology of Sulphides*, Geomicrobiology Science Publishers, Enfield, New Hampshire, 2010, pp. 101–137.
- [8] Y. Cheng, Z. Guo, X. Liu, H. Yin, G. Qiu, F. Pan, H. Liu, H. Liu, The bioleaching feasibility for Pb/Zn smelting slag and community characteristics of indigenous moderate-thermophilic bacteria, *Bioresour. Technol.* 100 (2009) 2737–2740.
- [9] H.L. Ehrlich, Beginnings of rational bioleaching and highlights in the development of biohydrometallurgy: a brief history, *Eur. J. Mineral Process. Environ. Protect.* 4 (2004) 102–112.
- [10] F. Gerayeli, F. Ghojavand, S.M. Mousavi, S. Yaghmaei, F. Amiri, Screening and optimization of effective parameters in biological extraction of heavy metals from refinery spent catalysts using a thermophilic bacterium, *Sep. Purif. Technol.* 118 (2013) 151–161.
- [11] A.A. Baba, F.A. Adekola, R.F. Atata, R.N. Ahmed, S. Panda, Bioleaching of Zn(II) and Pb(II) from sphalerite and galena ores by mixed culture of acidophilic bacteria, *Trans. Nonferrous Metals Soc. China* 21 (2011) 2535–2541.
- [12] S. Ilyas, R. Chi, J.C. Lee, H.N. Bhatti, One step bioleaching of sulphide ore with low concentration of arsenic by *Aspergillus niger* and taguchi orthogonal array optimization, *Chin. J. Chem. Eng.* 20 (2012) 923–929.
- [13] M.X. Liao, T.L. Deng, Zinc and lead extraction from complex raw sulfides by sequential bioleaching and acidic brine leaching, *Miner. Eng.* 17 (2003) 17–22.
- [14] Z. Manafi, H. Abdollahi, O.H. Tuovinen, Shake flask and column bioleaching of pyritic porphyry copper sulphide ore, *Int. J. Miner. Process.* 119 (2013) 16–20.
- [15] M. Rehman, M. Ahman-Anwar, M. Iqbal, K. Akhtar, A. Mukhtar-Khalid, M.A. Ghauri, Bioleaching of high grade Pb–Zn ore by mesophilic and moderately thermophilic iron and sulphur oxidizer, *Hydrometallurgy* 97 (2009) 1–7.
- [16] D.K. Xia, C.A. Pickles, Kinetics of zinc ferrite leaching in caustic media in the deceleratory period, *Miner. Eng.* 12 (1999) 696–700.
- [17] H. Abdollahi, S.Z. Shafaei, M. Noaparast, Z. Manafi, N. Aslan, Bio-dissolution of Cu, Mo and Re from molybdenite concentrate using mix mesophilic microorganism in shake flask, *Trans. Nonferrous Metals Soc. China* 21 (2013) 213–230.
- [18] S.P. Barik, K.H. Park, P.K. Parhi, J.T. Park, C.W. Nam, Extraction of metal values from waste spent petroleum catalyst using acidic solutions, *Sep. Purif. Technol.* 101 (2012) 85–90.
- [19] H. Tributsch, Direct versus indirect bioleaching, *Hydrometallurgy* 59 (2001) 177–185.
- [20] L. Xingyu, W. Biao, C. Bowei, W. Jiankang, R. Renman, Y. Guocheng, W. Dianzuo, Bioleaching of chalcocite started at different pH: response of the microbial community to environmental stress and leaching kinetics, *Hydrometallurgy* 103 (2010) 1–6.
- [21] W. Xin, C. Srinivasakannan, D. Xin-hui, P. Jin-hui, Y. Da-jin, J. Shao-hua, Leaching kinetics of zinc residues augmented with ultrasound, *Sep. Purif. Technol.* 115 (2013) 66–72.
- [22] H. Deveci, A. Akcil, I. Alp, Bioleaching of complex zinc sulphides using mesophilic and thermophilic bacteria: comparative importance of pH and iron, *Hydrometallurgy* 73 (2004) 293–303.
- [23] W. Sand, T. Gehrke, P.G. Jozsa, Shippers, A. (Bio) chemistry of bacterial leaching-direct vs. indirect bioleaching, *Hydrometallurgy* 59 (2001) 159–175.
- [24] K.H. Park, D. Mohapatra, B.R. Reddy, A study on the acidified ferric chloride leaching of a complex (Cu–Ni–Co–Fe) matte, *Sep. Purif. Technol.* 51 (2006) 332–337.
- [25] M.J. Leahy, M.P. Schwarz, Modeling jarosite precipitation in isothermal chalcocite bioleaching columns, *Hydrometallurgy* 98 (2009) 181–191.
- [26] S. Chen, J. Lin, Enhancement of metal bioleaching from contaminated sediment using silver ion, *J. Hazard. Mater.* 161 (2009) 893–899.
- [27] A.L. Juarez, N.G. Arenas, R.E.R. Santillan, Electrochemical behavior of massive chalcocite bioleached electrodes in presence of silver at 35 °C, *Hydrometallurgy* 83 (2006) 63–68.
- [28] R. Romero, A. Mazuelos, I. Palencia, F. Carranza, Copper recovery from chalcocite concentrates by the BRISA process, *Hydrometallurgy* 70 (2003) 205–215.
- [29] A. Deniz Bas, H. Deveci, E.Y. Yazici, Treatment of manufacturing scrap TV boards by nitric acid leaching, *Sep. Purif. Technol.* 130 (2014) 151–159.
- [30] M. Wang, Y. Zhang, T. Deng, K. Wang, Kinetic modeling for the bacterial leaching of chalcocite catalyzed by silver ions, *Miner. Eng.* 17 (2004) 943–947.
- [31] M.P. Silverman, D.G. Lundgren, Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. I. An improved medium and harvesting procedure for securing high cell yields, *J. Bacteriol.* 77 (1959) 642–647.
- [32] Z. Wei-min, G.U. Shi-fei, Catalytic effect of active carbon on bioleaching of low-grade primary copper sulfide ores, *Trans. Nonferrous Met. Soc. China* 17 (2007) 1123–1127.
- [33] D.F. Haghshenas, B. Bonakdarpour, E. Keshavarz, B. Nasernejad, Optimization of physicochemical parameters for bioleaching of sphalerite by *Acidithiobacillus ferrooxidans* using shaking bioreactors, *Hydrometallurgy* 111–112 (2012) 22–28.
- [34] D.E. Rawlings, Heavy metal mining using microbes, *Annual Rev. Microbiol.* 56 (2002) 65–91.
- [35] T. Rohwerder, T. Gehrke, K. Kinzler, W. Sand, Bioleaching review part A: progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation, *Appl. Microbiol. Biotechnol.* 63 (2003) 239–248.
- [36] H.R. Watling, The bioleaching of sulphide minerals with emphasis on copper sulphides – A review, *Hydrometallurgy* 84 (2006) 81–108.
- [37] M. Boon, M. Snijder, G.S. Hansford, J.J. Heijnen, The oxidation kinetics of zinc sulphide with *T. ferrooxidans*, *Hydrometallurgy* 48 (1998) 171–186.
- [38] M. Boon, J. Heijnen, Chemical oxidation kinetic of pyrite in bioleaching process, *Hydrometallurgy* 48 (1998) 98–111.
- [39] A. Akcil, S. Koldas, Acid Mine Drainage (AMD): causes, treatment and case studies, *J. Cleaner Product.* 14 (2006) 1139–1145.
- [40] D. Li, Y. Y. Niu, M. Fan, D. Xu, P. Xu, Focusing phenomenon caused by soil conductance heterogeneity in the electrokinetic remediation of chromium (VI)-contaminated soil, *Sep. Purif. Technol.* 120 (2013) 52–58.
- [41] A. Luptakova, S. Ubaldini, E. Macingova, P. Fornari, V. Giuliano, Application of physical–chemical and biological–chemical methods for heavy metals removal from acid mine drainage, *Process Biochem.* 47 (2012) 1633–1639.

- [42] F. Macias, M.A. Caraballo, J.M. Nieto, T.S. Rotting, C. Ayora, Natural pretreatment and passive remediation of highly polluted acid mine drainage, *J. Environ. Manage.* 15 (2012) 93–100.
- [43] R. Jamshidi Gohari, W.J. Lau, T. Matsuura, A.F. Ismail, Fabrication and characterization of novel PES/Fe–Mn binary oxide UF mixed matrix membrane for adsorptive removal of As(III) from contaminated water solution, *Sep. Purif. Technol.* 118 (2013) 64–72.
- [44] Y. Sun, X. Xiong, G. Zhou, C. Li, X. Guan, Removal of arsenate from water by coagulation with in situ formed versus pre-formed Fe(III), *Sep. Purif. Technol.* 115 (2013) 198–204.
- [45] S. Ghassa, Z. Boruomand, M. Moradian, H. Abdollahi, A. Akcil, Microbial dissolution of Zn–Pb sulphide minerals using mesophilic iron and sulphur oxidizing acidophiles, *Mineral Processing and Extractive Metallurgy Review* (2015) in press.