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Full Length Research Paper

Galena biooxidation by moderate sulphur bacteria

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Bioremediation is a simple and effective technology for metal extraction from low-grade contaminated soils and mineral concentrates. Metal remove from sulfide minerals is based on the activity of mesophilic and chemolithotrophic bacteria, mainly *Acidithiobacillus ferrooxidans* which convert insoluble metal sulfides into soluble metal sulfates. In this study bioremediation experiments carried out in 1 L Erlenmeyer flasks containing 300 ml basal medium of *A. ferrooxidans* and 5% (w/v) PbS with 45 and 75 meshes and also this condition repeated for *Acidithiobacillus thiooxidans*. The results showed that A*. ferrooxidans* had grown on the galena and obtained energy from it. Also, the galena was oxidized to form lead sulfate. The most important species for oxidizing galena concentrate showed *A. ferrooxidans,* because these species were more effective than *A. thiooxidans* in our bioremediation experiments. Anglesite ($PbSO_4$) was the important product of the galena bacterial oxidation. In these experiments the highest quantity of dissolute lead was 34% approximately in *A. ferrooxidans* cultures. The low solubility of lead sulphate indicated that this process is not commercially feasible for the recovery of lead on mines. In view of these results, bioremediation appears to have some potential for remediation of Pb contaminated soils.

Key words: Galena, *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, Ganat Marvan.

INTRODUCTION

There are many processes developed for remediation elements of its ores. Recently, bioleaching has been proposed for decontamination of solid wastes containing toxic metals such as sludge, soil, and sediment (Bosecker, 2001). Bioleaching is considered to be an efficient and an ecological friendly process commonly used by the miners as an alternative method to roasting or smelting, especially when there are lower concentrations of metal in the ore. This technique uses living organisms in order to degrade or transform contaminants into their less toxic forms (Sheela and Papinazath, 2003). One of bioremediation type is bioleaching that is the biological conversion of an insoluble metal compound into a water soluble form by microorganisms such as sulphur oxidizing bacteria (Mohd et al., 2009). The advantage of this technique includes low cost, high efficiency, and environment friendliness. The bacteria act as a catalyst to accelerate the natural processes inside the ore. The particular bacteria use a chemical reaction known as "Oxidation reaction" to convert metal sulphide crystals into sulphates and sheer metals.The main microbial species associated with the bioleaching process are *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. Bioleaching involves chemical and biological reactions. This study based on the activity of biological reactions of two microorganisms such as *A. ferrooxidans* and *A. thiooxidans.* Both microorganisms are aerobic and autotrophic bacteria, and carbon requirements are fulfilled by CO_2 from the atmosphere (Trivedi and Tsuchiya, 2003). The effectiveness of bioleaching is dependent on the physical, chemical and biological factors (Bosecker, 1997; Chen and Lin, 2001). Sulphidic ores such as sphalerit and galena are trophic resources of sulphuric bacteria. Zinc galena containing extremely

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Table 1. Constitutions of bioleaching concentrate from Ghanat Marvan mine.

Ni	S	Fe	Co	Su	Pb	Zn	LOI
0.1468%	34.23%	28.67%	0.76%	1.7%	16.34%	14.754%	3.3992%

high concentrations of lead remained without any management at the wide part of Kerman Province in Iran. Thus the objectives of the present study were to investigate the extraction of Pb metal from soil by bioleaching and identify the microbial and chemical factors associated with the bioleaching process.

MATERIALS AND METHODS

Microorganisms

In this study we use *A. ferrooxidans* and *A. thiooxidans* as moderate sulphur bacteria. *A. ferrooxidans* is an obligate autotrophic, and derives energy from the oxidation of pyrite (Qiu et al., 2005; Salari et al., 2008), various sulfur compounds (Amini et al., 2009) Sphalerite (Kavuri et al., 2009) and other sulphidic ores. This bacterium is too N_2-fixation (Mackintosh, 1978; Norris et al., 1995). *A. ferrooxidans* is the most studied metal sulfide oxidizing organism (Semenza et al., 2002; Schippers, 2004) and its strains have been often isolated from soil, waste and sludge of metals mines and acidic environments (Das et al., 1989). *A. thiooxidans* grows obligately autotrophically with various sulfur compounds, e.g. elemental sulfur (Suzuki, 1965), thiosulfate (Nakamura et al., 2001) and tetrathionate (Meulenberg et al., 2002). *A. thiooxidans* strains have been isolated from different acidic environments such as soil, sulfur deposits, and mine waste.

Sample preparing

The main lead concentrate is galena (lead sulfide), which is commonly found together with sphalerit. Lead concentrate were collected from Ganat Marvan lead mine. This mine lies in 25 Km north east of Baft in Kerman Province with the coordination (29° 20' 40″N, 56° 46' 56″ E). Concentrate were dried at lab temperature and sieved using 45 and 75 mesh sieve. The concentrate was analyzed by X-ray (XMF 104) in Table 1.

In preparation for the experiment the bacteria have been cultivated in a modified 9K medium. 9K medium has got the following composition: $(NH4)_2$ SO_4 3.0 g/L, KCl 0.1 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, Ca $(NO_3)_2$0.01 g/L, K_2 HPO_4 0.5 g/L, $FeSO_4 \cdot 7H_2O$ 9.84 g/L (Qiu et al., 2005). 2 M H_2SO_4 solutions were applicant to obtaining pH 2 medium. Cells were cultured at 30°C in Erlenmeyer flasks. The flasks were incubated on a rotary shaker at 180 rpm.

Bioleaching experiments

The bioleaching experiments were carried out in 1 L Erlenmeyer flasks. The flasks were filled with 300 ml of modified 9K medium and 5% (w/v) of concentrate and inoculated with 5% of *A. ferrooxidans* and *A. thiooxidans.* In the case of *A. thiooxidans* inoculation, 1 g of elemental sulfur was added to the flasks. The initial pH of the slurries was adjusted to 2.0 with H_2SO_4. All experiments were done and carried out in rotatory shaker at 180 rpm and 30°C for 45 days. Dissolved Pb concentrations were determined using atomic absorption spectrometer (Varian Spectra AA 220).

RESULTS AND DISSCUSION

The results showed that element sulphuric have high level in this concentrate. Pb is 16.34% and zinc is 14.754%. The sulphur is 34.23% thus the high amount of compounds in this concentrate are sulphidic ores mainly zinc sulphid and lead sulphide. Lead and zinc sulphide are to tropically course of moderate sulphur bacteria. The results of bioleaching experiments showed *A. ferrooxidans* has been adapted to the lead sulphide as the energy source and oxidized to form lead sulfate. The microbial bioleaching of the insoluble lead sulfides produces lead sulfate (anglesite), which also has a very low solubility. The most important species for oxidizing lead sulphide concentrate showed *A. ferrooxidans,* because these species was more effective than *A. thiooxidans* in our bioremediation experiments and could occur naturally in sludge of lead mines. In these experiments the highest quantity of dissolute lead was 34% approximately in *A. ferrooxidans* cultures. The low solubility of lead sulphate indicated that this process is not commercially for the recovery of lead on mines. Although lead is toxic for most microorganisms used in bioleaching, this effect is reduced due to the low solubility of the product. One of the beneficiation of bioleaching lead sulphidic is in complex ores, such as concentrates of solubilization of copper, nickel and zinc, which often occurs with galena where lead remains in the residue (Figure 1). In view of these results, bioremediation appears to have some potential for remediation of Pb contaminated soils not in mine recovery.

In comparison of other article in these fields Da Silva et al. (2003) report that during bioleaching processes galena was selectively oxidized to anglesite. Chemical leaching of residues obtained in the bioleaching of complex sulfides containing sphalerite and galena have been carried out to recover lead – using chemical leaching (Liao and Deng, 2004; Frías et al., 2002). In a consortium consisting of autotrophic and heterotrophic microorganisms was used in the bioleaching of metals from a copper-lead-zinc sulfide concentrate that copper and zinc extraction levels above 80%; in addition, bioleaching produced 83% galena oxidation from the concentrate (Tipre and Dave, 2004) (Figure 2). Particle size of concentrate showed positive correlation between particles and leached lead. This correlate is showed in Table 2.

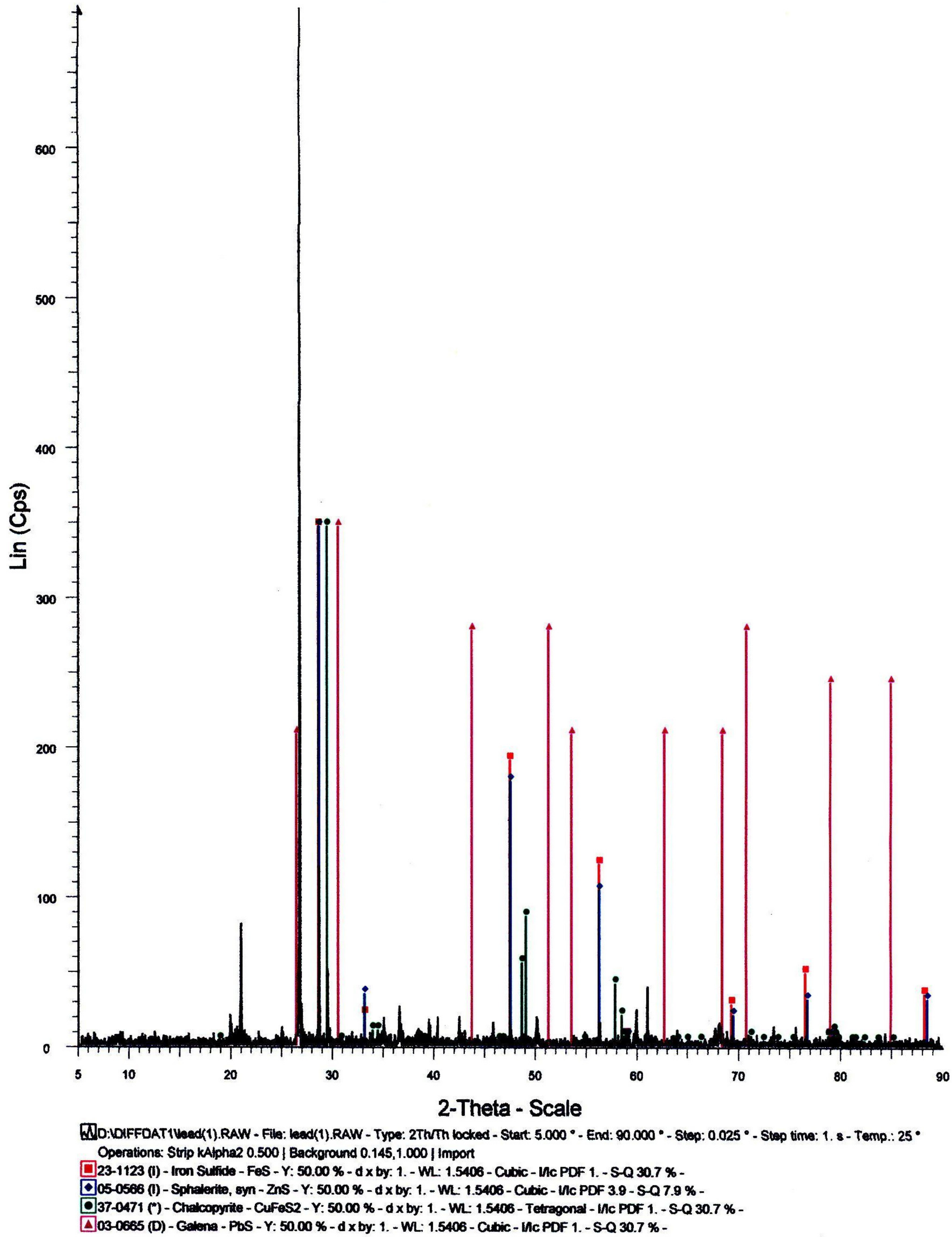
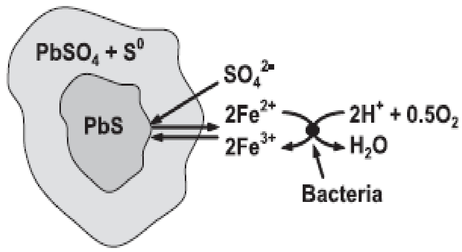


Figure 1. X-ray concentrate analysis of Ghanat Marvan is shown in Table 1.



$$PbS_{(s)} + 2Fe^{3+}_{(aq)} \rightarrow Pb^{2+}_{(aq)} + S^{0}_{(s)} + 2\ Fe^{2+}_{(aq)}$$

$$Pb^{2+}_{(aq)} + SO_4^{2-}{}_{(aq)} \rightarrow PbSO_{4(s)}$$

Figure 2. Schematic model composition of sulphur and angelesit indirect mechanism of bioleaching of galena (Pacholewska, 2004; Da Silva, 2004b).

Table 2. Correlations effect of particle size on the bioleaching of galena.

Data	Stril	PS<45	45<PS<75	PS>75
Stril	1	0.809(**)	0.803(**)	0.785(**)
PS<45	0.809(**)	1	0.995(**)	0.952(**)
45<PS<75	0.803(**)	0.995(**)	1	0.956(**)
PS>75	0.785(**)	0.952(**)	0.956(**)	1

*, **Correlation is significant at the 0.01 and 0.05 level (2-tailed).

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