BIO-STABILIZATION OF ARSENIC IN SOLID WASTES OBTAINED BY LIME TREATMENT OF BIOLEACHING LIQUORS

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ABSTRACT

The feasibility of biological stabilization of arsenic-containing bioleach liquors was evaluated at laboratory scale. The principle of the proposed process is to oxidize the As(III) present in the liquor using As(III)-oxidizing bacteria, in order to obtain a final solid residue containing mainly the more stable As(V) form. An As(III)-oxidizing consortium named CAsO1, selected by BRGM, was used to perform experiments with real liquors from bioleaching of gold-bearing concentrates. In a first experiment, it was decided to increase the pH of the liquor and incubate the resulting solids with CAsO1. Ca(OH)2 was used to increase pH. Two pH values were chosen: pH 4 and pH 7. Significant differences were observed between inoculated and not inoculated systems at pH 4, but not at pH 7. The more stable solids were obtained at pH 4 in the inoculated systems and the As(III) concentration in the solids was 40% lower in the inoculated than in the not inoculated systems. At pH 7, the solids phases of inoculated and not inoculated systems were not significantly different. In a second experiment, bacteria were injected directly in the reactor used for lime treatment, during the course of iron precipitation. This biological treatment allowed to decrease the As(III)/As(V) ratio in the resulting solids by 70%.

1. INTRODUCTION

Biological As(III)-oxidation may be useful for the treatment of effluents produced during the bioleaching of gold-bearing sulfidic concentrates. The pulp resulting from bioleaching often contains more than 1 g l^{-1} of As(III) and As(V). The effluents are treated by lime addition, resulting in removal of metals as hydroxide and jarosite precipitates. Arsenic co-precipitates with these compounds. However, the quality of the resulting solid wastes depends on the respective proportions of As(III) and As(V). Leaching tests showed that the stability of the waste increases with As(V) concentration in the initial solution (Grossin C., 1994). Thus, if some bacteria were able to increase As(V) concentration in the solution before lime treatment, the mobility of arsenic in the final solid waste would be decreased. However, the pH of the bioleaching solution is very low (pH < 2), and most of the As(III)-oxidising bacteria that were isolated and characterised so far are not true acidophiles. *Thiomonas arsenivorans* can grow at pH 2.5 (Battaglia-Brunet et al., 2006), but the maximum growth rate is obtained between pH 4 and pH 7.5. Similar results were obtained with a *Thiomonas* strain isolated from Carnoules site (Duquesnes, 2004).BRGM proposed to evaluate at small laboratory scale the feasibility of biological stabilization of arsenic-containing bioleach liquors. The principle of the proposed process is to oxidize the As(III) present in the liquor using As(III)-oxidizing bacteria, in order to obtain a final solid residue containing mainly the more stable As(V) form. It may be possible to increase the pH of the effluent up to 2.5, a slightly lower value than that for iron precipitation, and try to apply As(III) oxidation to the resulting solution. At this pH, *Thiomonas* cells may be inhibited by iron or other metals, however it would be interesting to test such a process. Another possibility would be to submit the solids wastes resulting from iron hydroxides precipitation to biological oxidation. The bacteria may be able to oxidize the adsorbed As(III) into As(V), either in the liquid phase (through the liquid-solid equilibrium) or directly in the solids. The feasibility of these two processes was tested at small laboratory scale.

2. MATERIAL AND METHODS

Biological Treatment of Solids Resulting from Lime Treatment

A bioleach liquor resulting from bioleaching of Petiknas ore was kindly supplied by MINTEK. The main characteristics of this solution are given in Table 1.

Table 1. Characteristics of Petiknas bioleach liquor

pH	**1.08**
Total Fe	30.5 g/l
Fe(II)	0.935 g/l
Total As	13.5 g/l
As(III)	1.35 g/l

Two types of solids were prepared: (1) the pH4 solids resulted from increasing the pH of the bioleach liquor to pH4, and (2) the pH7 solids resulted from increasing the pH of the bioleach liquor to pH7.

One litre of bioleach liquor was placed in a mechanically stirred reactor and the pH was increased by adding lime Ca(OH)2 pulp. The respective mass of Ca(OH)2 necessary to increase the pH were 98 g for the pH4 solids and 125 g for the pH7 solids. The solids were recovered by filtration, and stored as wet solids at 7°C, avoiding contact with air. The loss of weigh by drying at 45°C, determined on a small sample, was 62.2% for the pH4 solids and 66.6% for the pH7 solids. The arsenic was nearly entirely precipitated with iron at pH4 and pH7. The final As concentration (total As) in the filtrate was 8.8 mg.l-1 at pH4 and 1.36 mg.l-1 at pH7.

The CAsO1 population, containing Thiomonas arsenivorans, was used as inoculum (Battaglia-Brunet et al., 2002 and 2006), and prepared using the CSM medium (autotrophic) with 100 mg.l-1 As(III). Experiments were performed in sterile erlenmeyer flasks containing 60 g of wet solids and 90 ml of CSM medium without added As(III). For each pH, inoculated flasks and not-inoculated flasks (blanks) were prepared. In the inoculated flasks, 10 ml of inoculum were added. The solids were not sterilised. The pH of the liquid phases was the same as the pH of the solids wastes (pH4 or pH7).The flasks were incubated at 25°C in a reciprocal agitating incubator. All conditions were tested in triplicate. After 50 days of incubation, pulps were filtrated and the solids were rinsed with demineralised water and stored at 7°C under nitrogen.

The solutions in equilibrium with the solids were sampled at different incubation times. Samples were filtrated at 0.45 µm. As(III) and As(V) were separated on anionic resin (AG 1-X8©, Biorad, Hercules, CA, USA). Immediately after sampling, solutions were filtered at 0.45 µm, then 5 ml was passed through the column containing 2 g wet resin, and the column was rinsed with 5 ml demineralized water. The As(III)-containing solution was acidified with 50 µl concentrated HCl. As(V) was eluted with 2 x 5 ml HCL 1 M. Arsenic was quantified with an oven atomic absorption spectrophotometer (AAS, Varian, Palo Alto, CA, USA, T = 2050°C, 193.7 nm).

The As concentrations in the final solids were determined by the following method: extraction was performed using around 1.5 g of wet solids (corresponding to around 0.5 g of dry solids) + 15 ml H3PO4 0.5 M and ascorbic acid 0.2 M, 15 min microwaves 135W in a closed reactor. The analyses were performed by voltamperometric method for As(III) and ICP/AES for total As.

Biological Oxidation During Lime Treatment

A gold-bearing concentrate (Salsigne concentrate, Collinet-Latil, 1989, Grossin 1994), containing 40% arsenopyrite and 30% pyrite was bioleached at 35°C in batch, in a mechanically stirred and aerated glass bioreactor. The bioleaching conditions were the following: 10% solids, 0Km medium (Collinet-Latil, 1989), initial pH 1.75. The inoculum was a mesophilic bacterial mixed population maintained at BRGM for bioleaching experiments (D'Hugues et al., 2008). The final features of the bioleached pulp were the following: pH 1.11, redox potential 519 mV (Ref. Ag./AgCl), total As concentration 11 $g.l^{-1}$, total Fe concentration 11 $g.l^{-1}$. The bioleaching liquor was recovered by filtration of this bioleaching pulp in order to study the biological process of As(III) oxidation. Samples of bioleach liquor (1 l each) were treated in stirred bioreactors. One of the samples was classically treated with lime, without inoculation. Lime pulp, $Ca(OH)_2$, 200 $g.l^{-1}$ in demineralized water was used to increase the pH from 1.11 to 4.00 in order to precipitate Fe and As. In the case of the biological treatment, the following procedure was applied. pH was increased from 1.11 to 2.70 by lime addition. Then, 100 ml of a bacterial suspension was added to the pulp at each of the following pH steps: 2.70, 3.02, 3.11, 3.30, 3.52, 3.71, 3.89, and 4.00. The bacterial suspension was prepared with CAsO1 bacterial mixed population cultured in mineral medium MCSM containing 100 $mg.l^{-1}$ As(III) (Battaglia-Brunet et al., 2002). After each addition of bacterial suspension, the bioreactor was maintained 24 h at a stable pH value, under agitation, before lime is added in order to perform the next pH step and inoculation. As(III) concentration in the liquid phase of the bioreactor was determined by voltamperometric method. The final pulps were filtrated and the solids were stored under N_2 gas phase at 7°C. As speciation in the final solids was determined as described previously. Samples of solids from those

experiments were submitted to leaching experiments with demineralized water. Wet solids (25 g) and 100 ml demineralized water were reciprocally agitated in 250 ml Erlenmeyer flasks. At the beginning of the leaching test, pH was not adjusted and its value was in the range 3.6 to 3.9. Then, the pH was adjusted in some flasks at pH 5 by addition of NaOH 1M, in order to evaluate the influence of this parameter on the stability of As in solids. The As(III) and As(V) concentrations in liquid phases of those leaching experiments were determined after filtration at 0.45 µm and separation on anionic resins as described previously.

3. RESULTS AND DISCUSSION

Biological Treatment of Solids Resulting from Lime Treatment

The As(III) and As(V) concentrations in the liquid phases after 50 days of incubation, in presence or absence of CAsO1 bacterial population, are gathered in Table 2. The lowest total arsenic concentration is obtained at pH4 in presence of bacteria. These results show that the CAsO1 population was very active at pH4, even if this pH value is lower than the optimum pH for the bacteria. At pH4, the As(V) concentration was lower in the inoculated than in the not inoculated flasks. This result might be related to the Fe(II) oxidation activity of some bacteria present in CAsO1 population. As a matter or fact, the Fe(II) concentration was probably higher at pH4 than at pH7 in the liquid phase at the beginning of the experiment. The bacteria might have promoted the co-precipitation of residual As(V) and Fe at pH4. At pH7, the As(V) concentrations in the liquid phases of the inoculated and not inoculated experiments were not significantly different.

Table2. Arsenic concentrations in the liquid phases after 50 days of incubation

Condition	As(III) mg.l^{-1}	As(V) mg.l^{-1}	Total As mg.l^{-1}
Inoculated pH4	0.07	0.02	0.09
Not inoculated pH4	9.75	0.39	10.14
Inoculated pH7	< 0.01	0.34	0.34
Not inoculated pH7	< 0.01	0.48	0.48

The values of As(III) and total As extracted from the solids are given in Table 3. At pH4, the phosphate extraction allows to recover around 80% of total As, both from the initial and treated solids. The treated solids contain 40% less As(III) when incubated in presence of bacteria, compared to the not inoculated conditions. This result confirms that the bacterial activity allowed an efficient As(III) oxidation at pH4. The influence of bacterial activity on the solids-associated arsenic may be explained by two possible processes: (1) oxidation of As(III) in the liquid phase combined to adsorption <-> desorption equilibria, or (2) direct oxidation of As(III) onto the solids. Complementary experiments may allow to determine precisely which mechanism occurs, with the help of modeling.

Table 3. As(III) and total As extracted from the solids

		As(III) mg/g phosphate extracted	Total As mg/g phosphate extracted	% of As(III) phosphate extracted	Total As mg/g digestion of the solids	Phosphate extracted/total extracted ratio
pH4	Initial solids	10	65	15 %	77	84%
	Inoculated	6	62	10%	80	77%
	Blank	11	62	17%	80	78%
pH7	Initial solids	9	67	13%	69	97%
	Inoculated	4	47	9%	72	65%
	blank	5	45	10%	72	62%

At pH7, the phosphate extraction allowed to recover nearly 100% of total As from the initial solids, but only 65% of total As from treated solids. A physico-chemical phenomenon may have modified the form of solids-associated As during incubation at pH7, rendering it more resistant to phosphate extraction. The presence of phosphate in the liquid medium (6.5 mM) may have played a role in this phenomenon that remains to be determined. As(III) represents 10% of the final phosphate-extracted arsenic, both in the inoculated and in the not-inoculated conditions. There is no significant difference between inoculated and not-inoculated conditions. This result is in accordance with the analyses of the final liquid phases.

Biological Oxidation During Lime Treatment

The behaviour of As(III) concentration in the liquid phases of the two reactors, vs. pH, classical and biological treatment, is given in Figure 1.

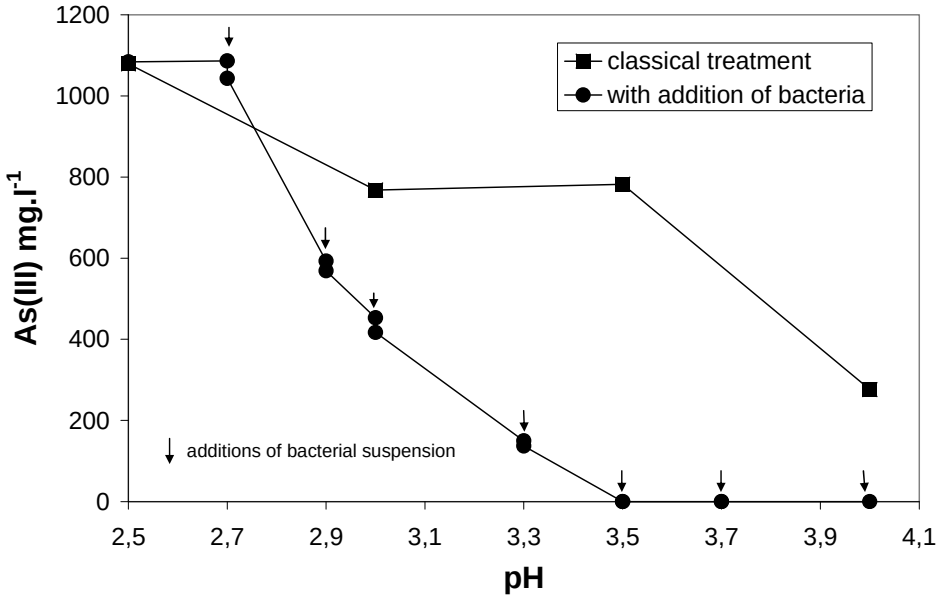

Figure 1. Evolution of As(III) concentration in the liquid phase during the lime treatment of a bioleach liquor with or without addition of As(III)-oxidizing bacteria.

The successive additions of bacterial suspension induced a decrease of As(III) concentration in the liquid phase of the inoculated reactor. This effect was already detectable between pH 2.7 and pH 2.9. As(III) oxidation is complete at pH 3.5 during the biological treatment whereas at the same pH As(III) concentration was 800 $mg.l^{-1}$ in absence of bacteria. Final As(III) concentration in the solids obtained through classical lime treatment was 54 $mg.g^{-1}$ (per g of dried solids, analytical standard error 5 $mg.g^{-1}$). In the solids obtained through biological oxidation process, the final As(III) concentration was 16 $mg.g^{-1}$ (per g of dried solids, analytical standard error 0.4 $mg.g^{-1}$). The biological oxidation of As(III) during lime treatment allowed to decrease the As(III) concentration in the final solids by 70%. Thus, biological oxidation during lime treatment was more efficient than biological oxidation of the solids after lime treatment. The results of the leaching tests performed with the final solids are given in Table 4. The solids obtained using the biological oxidation lime treatment were clearly more stable than the solids obtained through the classical lime treatment. As leached from the solids of the biological treatment is mainly in the As(V) form, whereas mainly As(III) was leached from the solids obtained with the classical lime treatment.

Table 4. Results of leaching experiments applied to solids previously submitted to biological oxidation during lime treatment or classical lime treatment.

	As		
Contact time	**24 h without pH adjustment (3.6<pH<3.9)**	**1 month without pH adjustment (3.6<pH<3.9)**	**1 month at pH 5**
Biological oxidation during lime treatment	As(III) 0.28 $mg.l^{-1}$ As(V) 0.29 $mg.l^{-1}$	As(III) 0.22 $mg.l^{-1}$ As(V) 0.89 $mg.l^{-1}$	As(III) 0.01 $mg.l^{-1}$ As(V) 0.31 $mg.l^{-1}$
Classical lime treatment	As(III) 151 $mg.l^{-1}$ As(V) 2.47 $mg.l^{-1}$	As(III) 173 $mg.l^{-1}$ As(V) 0.33 $mg.l^{-1}$	As(III) 38 $mg.l^{-1}$ As(V) 0.36 $mg.l^{-1}$

4. CONCLUSION – PERSPECTIVES

The results of the present experiments suggest that the stabilization of solids wastes from bioleach liquors using acido-tolerant As(III)-oxidizing bacteria is technically feasible. The efficiency of As(III) oxidation was clearly demonstrated at pH values as low as 2.9, whereas the optimum growth pH of CAsO1 population was pH6. The present results also showed for the first time that the ability of CAsO1 population to oxidize As(III) was not inhibited by the diverse heavy metals present in the bioleaching liquor. The biological oxidation was more efficient when applied during the course of lime treatment, before the end of iron precipitation and arsenic co-precipitation, than when applied after the lime treatment step. In the present study, the addition of bacterial population was initiated when the pH of the pulp was 2.7. It may be interesting to test the introduction of As(III)-oxidizing bacteria at lower pH, when even more As(III) remained in solution. The incubation time at each pH step also remains to be optimized, however the results of the present study are very encouraging and may serve as a basis for the development of original processes for the stabilization of bioleaching solid wastes containing arsenic among other applications.

5. REFERENCES

Battaglia-Brunet, F., Dictor, M.-C., Garrido, F., Crouzet, C., Morin, D., Dekeyser, K., Clarens, M., and Baranger, P. (2002) “An As(III)-oxidizing bacterial population: selection, characterization, and performance in reactors.” Journal of Applied Microbiology, **93**, 656-667.

Battaglia-Brunet, F., Joulian, C., Garrido, F., Dictor, M.C., Morin, D, Coupland, K., Johnson, D.B., Hallberg, K.B., and Baranger P. (2006) “Oxidation of arsenite by Thiomonas strains and characterization of Thiomonas arsenivorans sp. nov.” Antonie Van Leeuwenhoek International Journal, **89**, 99-108

Collinet-Latil, M.-N. (1989) « Lixiviation bactérienne par Thiobacillus ferrooxidans et Thiobacillus thiooxidans d’un concentré de flottation arsénopyriteux aurifère réfractaire à la cyanuration directe »., PhD Thesis, Université de Provence Aix-Marseille I, spécialité Biologie cellulaire et microbiologie.

D’Hugues, P., Joulian, C., Spolaore, P., Michel, C., Morin, D. (2008) “Continuous bioleaching of a pyrite concentrate in stirred reactors: population dynamics and exopolysaccharide production vs. bioleaching performance.” Hydrometallurgy, 94 : 34-41.

Duquesne, K. (2004) “Rôle des bactéries dans la bioremédiation de l’arsenic dans les eaux acides de drainage de la mine de Carnoules”. PhD Thesis, Université de la Méditerranée, Aix-Marseille II.

Grossin, C. (1994) « Etude de la morphologie et de la stabilité des arséniates de fer synthétiques. » PhD Thesis, Université d’Orléans.

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