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Małgorzata PACHOLEWSKA[1] and Beata CWALINA[2]

RESISTANCE OF *Acidithiobacillus ferrooxidans* TO As(III) AND Sb(III) IONS

OPORNOŚĆ *Acidithiobacillus ferrooxidans* WOBEC JONÓW As(III) ORAZ Sb(III)

Abstract: The influence of the Sb(III) and As(III) ions on metabolic activity of acidophilic bacteria *Acidithiobacillus ferrooxidans* isolated from the zinc-lead post-flotation tailings have been studied. It may be stated that these bacteria feature high resistance to the As(III) ions and lack of the Sb(III) ions tolerance. Bacteria tolerate the As(III) at concentrations up to 50 mg/dm^3 but do not tolerate Sb(III) even at concentration of 10 mg/dm^3. Thus, the strain being tested cannot be used in processes of the feed electrolyte treatment and cleaning.

Keywords: *Acidithiobacillus ferrooxidans,* Fe(II) oxidation, resistance to As(III) and Sb(III)

The influence of As(III) and Sb(III) ions on metabolic activity of iron-oxidizing bacteria *Acidithiobacillus ferrooxidans* cultured in liquid media stimulating their growth, have been investigated. The bacteria tested were isolated from surface layer of the zinc-lead post-flotation tailings originating from the mining-metallurgy plant ZGH Boleslaw S.A. [1]. Against the popular belief that arsenium and antimonium compounds are toxic to living organisms, it is suggested that these elements-rich natural environments are favourable to many organisms adaptation leading to their resistance to toxic substances [2, 3]. Such effect has been observed in the gold mines where the arsenium-containing minerals are usually present. As contrasted with the arsenium, the antimonium effect on living organisms is not fully recognised as yet [4, 5].

The investigated waste materials acquired from ZGH Boleslaw S.A. are composed mainly of the Ca and Mg minerals (dolomites). They also contain some quantity of heavy metals including Fe, Zn, Pb, Cd, Tl, Cu, Ag, Ni, As, and Sb [6]. Their percentage

[1] Department of Metallurgy, Silesian University of Technology, ul. Z. Krasińskiego 8, 40–019 Katowice, Poland, email: Malgorzata.Pacholewska@polsl.pl

[2] Environmental Biotechnology Department, Silesian University of Technology, ul. Akademicka 2, 44–100 Gliwice, Poland, email: Beata.Cwalina@polsl.pl

in flotation tailings is varying throughout the tailings pond and dependent on storage time (Table 1).

Table 1

Concentrations of As and Sb in flotation tailings from ZGH Boleslaw S.A.

Storage yard	A1 – the oldest part		A2 – the youngest part	
Depth	0.0 m	3.0 m	0.0	3.0 m
As and Sb concentrations [%]				
As	0.098	0.057	0.085	0.075
Sb	0.0063	0.0045	0.0052	0.0041

Total amounts (estimated in 1990s) of some toxic metals present in the tailing ponds are as follows: As-13105.7 Mg, Cd-1967.2 Mg, and Tl-664.9 Mg. Some amounts of As and Sb remain in the zinc-bearing raw materials from which they pass into electrolyte solution. Removal of As and Sb from this solution is carried out due to chemical oxidation of ferrous ions to Fe(III). It is suggested that the iron-oxidizing *A. ferrooxidans* bacteria indicating resistance to arsenium and antimonium ions could be useful in biological treatment of the feed electrolyte.

Materials and methods

The metabolic activity of acidophilic bacteria *A. ferrooxidans* strain B1 = SB isolated from post-flotation tailings [1] was investigated taking into account these bacteria iron-oxidizing ability. Experiments were carried out in glass bioreactors of 300 cm^3 volume, using synthetic culture medium 9K of Silverman and Lundgren (S/L) [7]. This medium contains [g/dm^3]: $NH_4)_2SO_4$ – 3.0; KCl – 0.1; $MgSO_4 \cdot 7H_2O$ – 0.5; $Ca(NO_3)_2$ – 0.01; K_2HPO_4 – 0.5; Fe(II) – 9.0 ($FeSO_4 \cdot 7H_2O$). The 100 cm^3 samples of 9K medium were inoculated with 2 cm^3 of the culture suspension containing bacteria in exponential growth phase. Solutions were aerated and mechanically stirred. Their temperature was maintained on level 25 ^oC. The Sb(III) and As(III) ions were introduced into 9K solution (pH ca 2.5) as standard water solutions containing respective ions at concentration of 1.0 g/dm^3, to obtain final concentrations (in 9 K solution) of 10, 20 and 50 mg/dm^3 being 10 times higher than those occurring in polluted industrial electrolyte. The standard solutions have been received from the analytical laboratory Boltherm, Bukowno. The standard As(III)-solution has been produced in Regional Verification Office, Lodz whereas the standard Sb(III)-solution has been produced in Tusnovics Instruments Sp. z o.o., Krakow. Solutions with bacteria as well as sterile control solutions without bacteria were investigated. The Fe(II) and Fe(III) concentrations were measured by a complexometric method with sulfosalicylic acid [8].

Results

The influence of Sb(III)

Changes in Fe(II) and Fe(III) concentrations in the 9K solution under the action of *A. ferrooxidans* strain B1 in the presence of Sb(III) ions have been presented in Figs 1–2 and in Table 2.

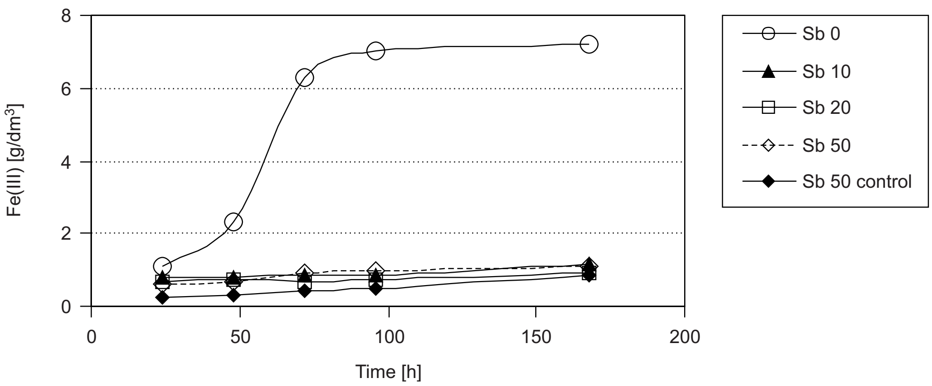

Fig. 1. The influence of Sb(III) mg/dm^3 on metabolic activity of the *Acidithiobacillus ferrooxidans* strain B1, reflected by increase in the Fe(III)-concentration in 9K solution

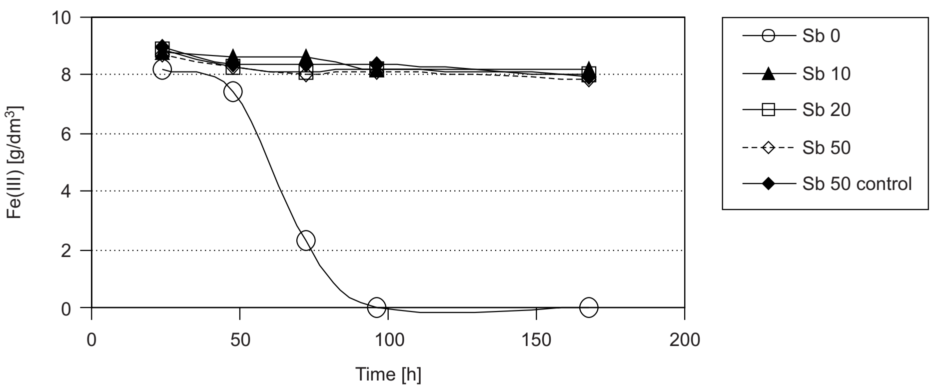

Fig. 2. The influence of Sb(III) mg/dm^3 on metabolic activity of the *Acidithiobacillus ferrooxidans* strain B1, reflected by decrease in the Fe(II)-concentration in 9K solution

From these results it may be concluded that: 1) Sb(III)-presence in 9K culture medium causes an inhibition of the iron-oxidizing activity in *A. ferrooxidans* strain B1; 2) *A. ferrooxidans* strain B1 indicates high susceptibility to Sb(III) ions even at their lowest concentration used (concentration of 10 mg/dm^3 was toxic for B1); 3) average rate of the Fe(III)-concentration increases due to metabolic activity of *A. ferrooxidans* strain B1 decreases about 6-times (from 42.8 to 7.1 mg/dm^3/d; Table 2) in the presence of Sb(III) ions at the lowest concentration used (10 mg/dm^3).

Table 2

The influence of Sb(III) and As(III) on metabolic activity of the *Acidithiobacillus* ferrooxidans strain B1, reflected by average rates of changes in Fe(II) and Fe(III)-concentrations in 9K solution during 168 hours

Supplement	$\Delta\downarrow$ Fe(II)		$\Delta\uparrow$ Fe(III)	
	mg/dm^3/d	mg/dm^3/h	mg/dm^3/d	mg/dm^3/h
Sb(III) [mg/dm^3]				
0.0	93.7*	3.90*	42.8	1.80
10.0	4.8	0.20	7.1	0.29
20.0	5.6	0.23	5.4	0.22
50.0	6.6	0.27	6.6	0.27
50.0 (control; sterile)	6.2	0.26	5.0	0.21
As(III) [mg/dm^3]				
0.0	93.7*	3.90*	42.8	1.80
10.0	93.7*	3.90*	43.7	1.82
20.0	93.7*	3.90*	45.7	1.90
50.0	93.7*	3.90*	44.1	1.84
0.0 (control; sterile)	7.9	0.33	6.6	0.27
50.0 (control; sterile)	7.0	0.29	4.6	0.19
As(III) 50 + Sb(III) 10 [mg/dm^3]	13.7	0.57	9.5	0.40

$\Delta\downarrow$ Fe(II), $\Delta\uparrow$ Fe(III) – mean rates of Fe(II)-concentration decrease or Fe(III)-concentration increase; * – the rate estimated after 96 hours.

The influence of As(III)

Changes in Fe(II) and Fe(III) concentrations in the 9K solution under the action of *A. ferrooxidans* strain B1 in the presence of As(III) ions have been presented in Figs 3–4 and in Table 2. From these results it may be pointed that: 1) *A. ferrooxidans* strain B1 indicated high tolerance and resistance to As(III) ions; 2) the As(III)-presence at concentration of 50 mg/dm^3 caused inconsiderable increase in the rate of Fe(II) ions bacterial oxidation; 3) simultaneous presence of both Sb(III) and As(III) ions in 9K liquid culture medium caused the decrease in metabolic activity of bacteria tested; average rate of the Fe(III)-concentration increase due to metabolic activity of *A. ferrooxidans* strain B1 decreased almost 5-times (from 44.1 to 9.5 mg/dm^3/d; Table 2) in the presence of Sb(III) and As(III) ions at the lowest concentrations used (10 mg/dm^3).

Presented results indicated that the *A. ferrooxidans* strain B1 possesses increased arsenic resistance. It is accepted that the arsenic resistance and metabolizing systems occur in bacteria in three patterns: the widely-found ars operon that is present in most

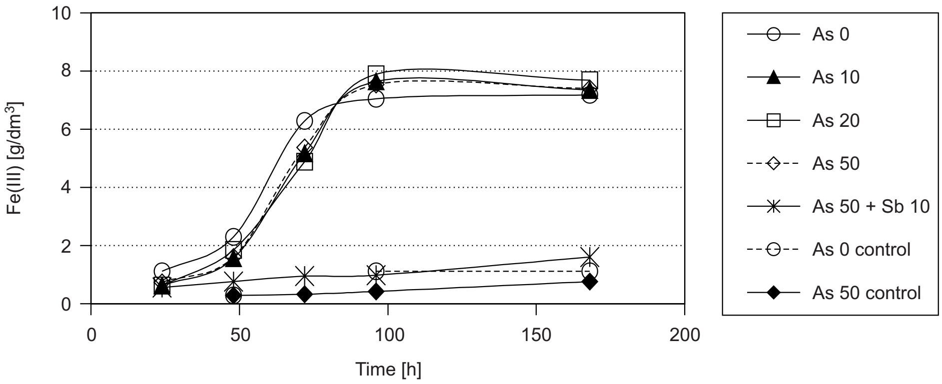

Fig. 3. The influence of As(III) mg/dm^3 on metabolic activity of the *Acidithiobacillus ferrooxidans* strain B1, reflected by increase in the Fe(III)-concentration in 9K solution

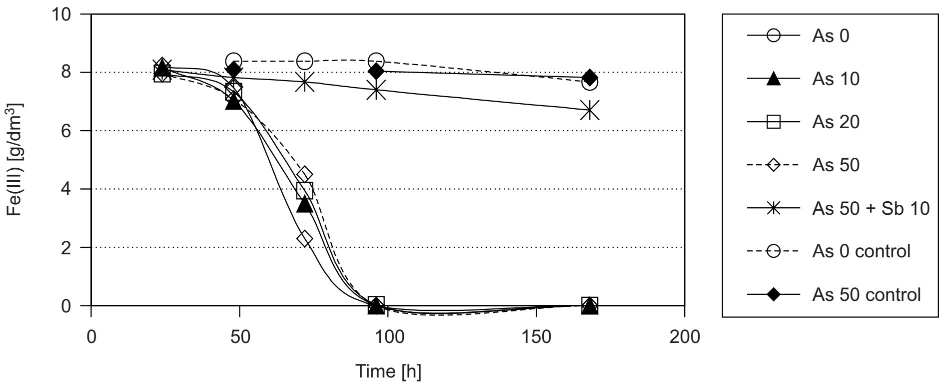

Fig. 4. The influence of As(III) mg/dm^3 on metabolic activity of the *Acidithiobacillus ferrooxidans* strain B1, reflected by decrease in the Fe(III)-concentration in 9K solution

bacterial genomes and many plasmids, the more recently recognized arr genes for the periplasmic arsenate reductase, and the aso genes for the periplasmic arsenite oxidase [10]. It has been shown that after long time (two years) of selection, *A. ferrooxidans* bacteria may become sufficiently resistant to the 13 g/dm^3 total arsenic in solution. The *A. ferrooxidans* strain B1 used in our experiments tolerate the As(III) at concentrations up to 50 mg/dm^3. Such a resistance level would be sufficient for this strain practical using in processes of the feed electrolyte treatment, but these bacteria are not antimony resistant. Taking into account that industrial electrolyte is usually a multi-component solution, in which many other impurities such as Cu(II), Cd(II), and Ag(I) ions (characteristics of the *A. ferrooxidans* strain B1 resistance to these ions have already been investigated and presented [9]), as well as the Sb(III) ions occur simultaneously with As(III) ions, it can be concluded that the *A. ferrooxidans* strain B1 is not useful for treatment the multi-component feed electrolytes originated in ZGH Boleslaw S.A., due to their high overall toxicity towards bacteria.

Conclusions

Basing on the investigations on the influence of the Sb(III) and As(III) ions on metabolic activity of acidophilic bacteria *A. ferrooxidans* strain B1 = SB, which have been isolated from post-flotation tailings resulting from processes of zinc-lead ores enrichment in the ZGH Boleslaw S.A., it may be stated that these bacteria feature high resistance to the As(III) ions and lack of the Sb(III) ions tolerance (resistance to). It has been demonstrated that bacteria tolerate the As(III) presence at concentrations up to 50 mg/dm^3 but do not tolerate Sb(III) even at concentration of 10 mg/dm^3. Thus, the strain being tested cannot be used in processes of the feed electrolyte treatment and cleaning.

Acknowledgements

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OPORNOŚĆ *Acidithiobacillus ferrooxidans* WOBEC JONÓW As(III) ORAZ Sb(III)

[1] Katedra Metalurgii, [2] Katedra Biotechnologii Środowiskowej
Politechnika Śląska

Abstrakt: Badano wpływ jonów Sb(III) i As(III) na aktywność metaboliczną kwasolubnych bakterii *Acidithiobacillus ferrooxidans*, izolowanych z odpadów poflotacyjnych rud cynku i ołowiu. Ustalono, że bakterie te wykazują dużą oporność na jony As(III) i brak tolerancji Sb(III). Bakterie tolerują As(III) w stężeniach do 50 mg/dm^3, ale nie tolerują Sb(III) nawet w stężeniu 10 mg/dm^3. Tak więc testowany szczep nie może być wykorzystany w procesach przeróbki i oczyszczania nadawy elektrolitu.

Słowa kluczowe: *Acidithiobacillus ferrooxidans*, utlenianie Fe(II), oporność na As(III) i Sb(III)