

Małgorzata PACHOLEWSKA\*

## **MICROBIAL LEACHING OF BLENDE FLOTATION CONCENTRATE USING *ACIDITHIOBACILLUS FERROOXIDANS* AND *ACIDITHIOBACILLUS THIOOXIDANS***

*Received March 2003, reviewed, and accepted May 15, 2003*

Bacterial leaching of blende flotation concentrate from Zakłady Górniczo-Hutnicze „Bolesław” was carried out using single cultures and mixed cultures of acidophilic sulphur- and iron- oxidizing bacteria *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans*. The accelerating effect of mixed cultures of bacteria on the metal leaching was amplified by correcting pH leaching medium to low values. The overall conversion of zinc exceeded 35-40%. The leaching rates of ZnS depended on redox potential, concentration of Fe(III)/Fe(II) and pH. X-ray analysis and electron scanning microscopy showed that in inoculated solution new solid phases: jarosite, elemental sulphur and anglesite were formed.

*Key words: blende flotation concentrate, microbial leaching, Acidithiobacillus thiooxidans, Acidithiobacillus ferrooxidans*

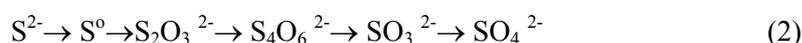
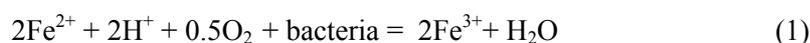
### INTRODUCTION

Extraction of zinc from sulphide minerals using iron- and sulphur oxidizing bacteria has been studied by many investigators (Konishi et al. 1992, Pistorio et al. 1994, Cwalina et al. 1994, Sanmugasunderam et al. 1988, Ahonen et al. 1995, Fowler et al. 1998, Boon et al. 1998) but the reported efficiency of bioleaching process was varying. It was found that semiindustrial tank leaching of zinc sulphide concentrates by *Acidithiobacillus ferrooxidans* resulted in zinc concentration in pregnant solution as high as 120 g/dm<sup>3</sup> and rates of release of zinc into the solution as fast as 1300 mg/dm<sup>3</sup>h (Torma 1988). A previous study (Gormely et al. 1975) showed that, while continuous microbiological leaching of Zn from ZnS concentrate was technically feasible, extraction levels tended to be rather low (range 11-72 %, average 45%). To

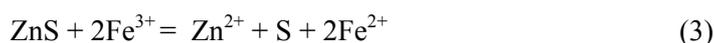
---

\* Politechnika Śląska, Katedra Metalurgii, 40-019 Katowice, ul. Krasińskiego 8, Poland,  
malg@polsl.katowice.pl

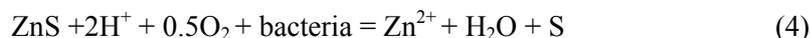
improve the recovery of metal, addition of the second reactor stage or separation of the unleached residue and recycling it back to the reactor were proposed. Lochmann and Pedlik (1995) stated, that during the chemical leaching of sphalerite concentrates in acidic ferric sulphate solution, the dissolution rate of zinc decreased with time and overall conversion has not exceeded 30%. They found, that the growing elemental sulphur layer on the surface of each particle was found to be the cause of this passivation. The microorganisms *Acidithiobacillus ferrooxidans* are able to oxidize ferrous ions and the reduced sulphur compounds (Orłowska and Gołąb 1990) and *Acidithiobacillus thiooxidans* are able to oxidize only reduced sulphur compounds, summarized by the global reaction (Skłodowska 1990, Cwalina 1994):



The product of oxidation of ferrous ions, ferric ions, is a strong oxidant that is capable of oxidizing sulfide materials. The overall leaching process occurs by bacterial oxidation referred to as indirect mechanism (Hansford and Vargas 2001) is:



or as direct interact with the mineral:



The new integral model described by (Sand et al. 2001) stated that the mechanism and biodegradation of sulphide minerals is determined by their structure. Sphalerite is degradable by iron (III) and proton attack. The dissolution proceeds via  $\text{H}_2\text{S}^{**}$  radical and polysulfides to elemental sulphur. The kinetics is mainly dependent on the concentration of Fe(III) and on the solubility product.

The objective of this study was to determine the efficiency of zinc extraction during bacterial leaching of blende-sphalerite flotation concentrate obtained from ZGH "Boleslaw" using a) single bacterial culture and b) mixed bacterial cultures of strains *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*.

## EXPERIMENTAL METHODS

### BLLENDE-FLOTATION CONCENTRATE

Blende flotation concentrate was supplied by ZGH "Boleslaw". The Zn-Pb ores from local deposits are concentrated in flotation plant "Olkusz-Pomorzany" with prior concentration in heavy liquids. Analysis of the concentrate and the particle size distribution of the concentrate were shown in Table 1. The major minerals present in

those samples were sphalerite (78%), galena (3%), with small amounts of pyrite (8%) marcasite (4%), dolomite and calcite (5%), hydrocerrusite (1%) and quartzite (1%). Concentrate samples containing different particle sizes were used in the experiments.

#### BACTERIA

The bacteria used were a strain of *Acidithiobacillus ferrooxidans* (F3-02) and a strain of *Acidithiobacillus thiooxidans* (T5-02), isolated from the source of mineral water coming from Głębokie and Łomnica (Nowy Sącz county), (Pacholewski and Pacholewska 2001). *Acidithiobacillus ferrooxidans* were harvested in a medium Silverman - Lundgren 9K (Silverman and Lundgren 1959) consisting of:  $(\text{NH}_4)_2\text{SO}_4$  - 3.0; KCl - 0.1;  $\text{K}_2\text{HPO}_4$  - 0.5;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  - 0.5;  $\text{Ca}(\text{NO}_3)_2$  - 0.01;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  - 44.2 g/dm<sup>3</sup>. To adapt the original strain to the ZnS concentrate as a solid substrate, the strain was subcultured in the modified 2K (2.0 g/dm<sup>3</sup> Fe(II)) liquid medium supplemented with 5% w/v ZnS concentrate. The bacteria *A. thiooxidans* were harvested in a Waksman-Joffe medium consisting of:  $(\text{NH}_4)_2\text{SO}_4$  - 0.2;  $\text{KH}_2\text{PO}_4$  - 3.0;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  - 0.5;  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$  - 0.25; S<sup>o</sup> element. 10.0 g/dm<sup>3</sup>. All cultures for leaching experiments were treated with 10 cm<sup>3</sup> of inocula in 300 cm<sup>3</sup> Erlenmeyer flasks containing 100 cm<sup>3</sup> of leaching medium (2K) with 5.0 g sphalerite concentrate. The volume of inoculum of single bacterial cultures was 10 cm<sup>3</sup> and the mixed culture was 5 cm<sup>3</sup> of *A. thiooxidans* and 5 cm<sup>3</sup> *A. ferrooxidans*. Experiments were started under exactly the same solution conditions of pH and redox potential of leaching medium in bacterial and control leaching.  $\text{NH}_4\text{Fe}(\text{SO}_4)_2$  (ferric ammonium sulphate) was added to initial control samples as a source of Fe(III). The pH of the medium was adjusted to 2.0 by adding  $\text{H}_2\text{SO}_4$ . The samples were placed in laboratory water bath-shaker with shaking 1 at 30 oscillation/min and amplitude 5. The temperature during leaching was maintained at 22-23°C. All the experiments were done three times.

#### ANALYSIS

To quantify the amount of zinc and iron dissolved in the leaching media, a 1 cm<sup>3</sup> sample was filtered and the filtrate was analyzed by an atomic absorption spectrophotometer (SOLAAR M6-UNICAM Atomic Absorption). 1 cm<sup>3</sup> solution was taken also to determine the concentration of ferrous ion by manganometric method using 0.002 M  $\text{KMnO}_4$  reagent. The precipitates after leaching were washed with ethanol to fix the bacteria on the surfaces of the mineral particles, after that they were dried and preserved for subsequent analysis. Total sulphur in solid residues and S-sulphate were analyzed by classical weight method. X-ray diffraction patterns for the precipitates were generated by exposing the dried samples to Cu - $\text{K}\alpha_1$  radiation at 40 kV, 35 mA. Scanning electron microscope HITACHI-4200 has been used to image the microscopic structural properties of probes.

## RESULTS AND DISCUSSION

## LEACHING BY SINGLE CULTURES

Figure 1 shows the results of the experiment in which the dissolved zinc concentration from sulphide flotation concentrates was determined in the 2K medium in the presence of different bacteria cultures. The results indicated that the zinc concentration in solution was higher in the presence of *Acidithiobacillus ferrooxidans* as compared to *Acidithiobacillus thiooxidans* and the control experiment. The maximum concentration of zinc leached in this way amounted to about 5.35 g/dm<sup>3</sup>. After reaching maximum concentration, zinc tended to reprecipitate in further leaching. The results of the control leaching experiments were similar to the results of bacterial leaching experiments but the dependency of the released zinc from the mineral in time did not come up to maximum. The total amount of zinc leached in 312 h (15 days) amounted to about 32.36 % with bacteria *A. ferrooxidans* and 21.82 % with *A. thiooxidans*, without 22.69% - Table 2.

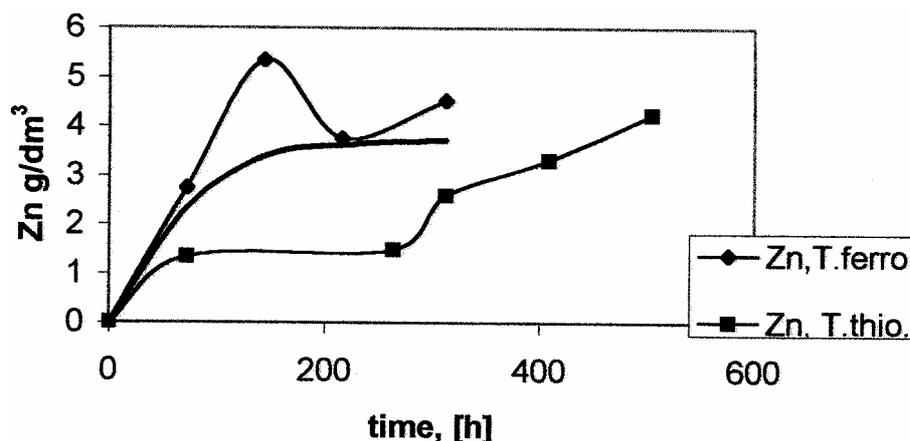


Fig.1. Effect of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* on leaching rate of sphalerite concentrate

## LEACHING BY MIXED CULTURES

Mixed cultures of *A. ferrooxidans* and *A. thiooxidans* generally showed increased leaching rates of zinc compared with single cultures (Fig. 2). Two-stage course of zinc dissolution reaction can be observed. Initially there was a constant growth of zinc concentration until it has reached the point where there was rapid change of reaction rate. The maximum concentration of zinc leached in this way was 10.28 g/dm<sup>3</sup> in 408 h (17 days). It was observed that after obtaining maximum, the zinc was reprecipitated and the concentration of Zn remained constant at the level of about 7.5g/dm<sup>3</sup> for further 768 h of leaching, showing passivation tendency, as stated by (Lochmann and Pedlik

1995). The observed tendencies reveal that zinc sulphide is most likely initially dissolved by the activity of the sulphur-oxidizing bacteria and then by iron- and sulphur-oxidizing bacteria. *A. thiooxidans* which was expected to produce sulphuric acid from sulphides, did not reduce strongly enough the acid requirement. It was necessary to add acid to the system. A solution of sulphuric acid was added in order to bring the pH to 2.0. The results of the second experiment showed in Fig.2, in which continuous control of pH leaching solution was applied, gave the similar level of dissolved zinc in 504 h of leaching - 8.28 g/dm<sup>3</sup>. However, in this case the rate of dissolution of zinc from sulphide concentrate increased continuously with time. It was observed that after start of leaching process (about 144 h) H<sub>2</sub>S gas was generated. The total amount of zinc leached in 768 h with mixed culture was 40.28% and 37.53% during 504 h in solution with correcting pH (Table 2).

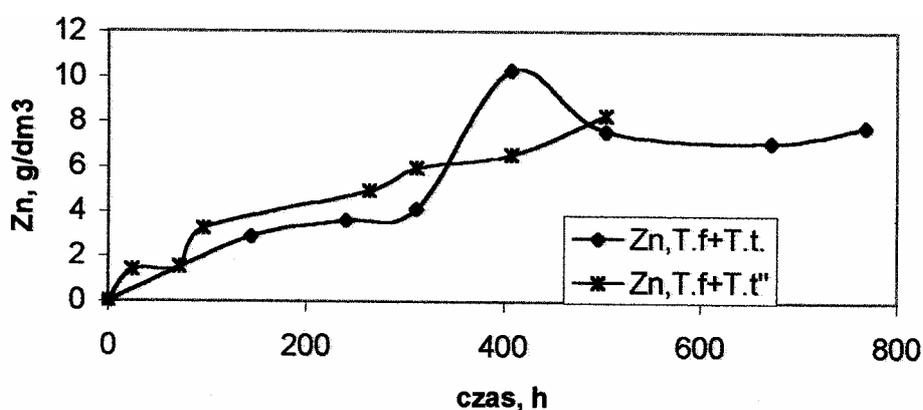


Fig.2 Effect of a mixed cultures of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* on leaching rate of sphalerite concentrate

Table 1. Compositions of blende (sphalerite) concentrate from Zakłady Górniczo-Hutnicze "Bolesław"

Compounds	[%]	Mean particle diameter [mm]	Weight fraction [%]
Zn	53.55	0.16	7.76
Pb	1.50	0.125	12.72
Cd	0.29	0.100	16.12
Stotal	31.3	0.063	23.16
S SO <sub>4</sub>	0.29	<0.063	40.22
S S	31.0		
MgO	0.98		
CaO	2.78		
SiO <sub>2</sub>	0.40		

Table 2. Composition of solid residues after bioleaching and control leaching

Sample	Zn [%]	Pb [%]	Fe [%]	CaO [%]	Cd [%]	S total [%]	S so4 [%]	Weight of residue [g]	Extract. of Zn [%]
ZnS1 <i>A. ferroox.</i>	48.31	1.64	6.33	0.97	0.27	31.84	1.73	3.7543	32.36
ZnS11 <i>A. thioox.</i>	53.93	1.49	4.40	1.47	0.29	30.91	1.24	3.8815	21.82
ZnS5 Mixed culture	50.46	1.52	4.98	1.38	0.29	30.91	1.48	3.1686	40.28
ZnS8 Mixed culture pH 2.0	49.70	1.57	6.05	1.28	0.27	32.23	1.85	3.3654	37.53
ZnS13 Control	56.00	1.52	4.39	0.78	0.30	31.06	0.81	3.6963	22.69

## pH EFFECT

Figure 3 presents summary results of pH changes of the solutions at leaching time of zinc sulphide concentrate in the environment of different bacteria cultures and in the control leaching. The lowest pH was observed (as it has been expected) in the solution with *A. thiooxidans* whereas the highest pH was observed in the solutions with *A. ferrooxidans*. In Fig. 3 the changes of pH before adjustment to constant value have been presented.

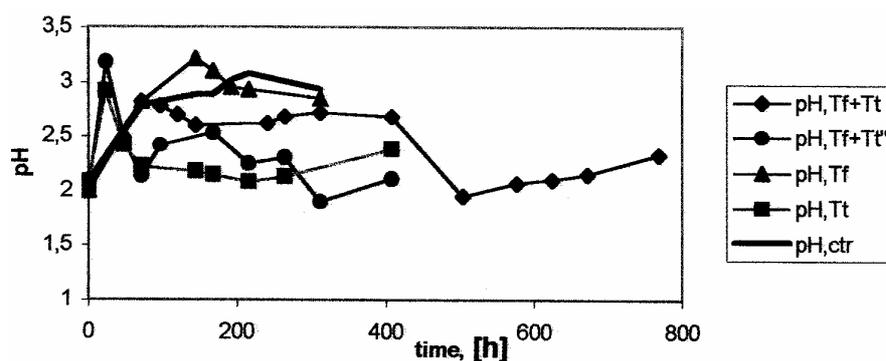


Fig. 3. pH evolution during leaching of blende concentrate in the presence of: *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* and mixed cultures. (") -adjusting solution to pH 2.0

## EFFECT OF Eh - REDOX POTENTIAL

The changes of redox potential in the course of time have been presented in Fig.4. It can be clearly seen that the most rapid growth of oxidizing properties can be noticed when the mixed culture has been used (*A. ferrooxidans* and *A. thiooxidans*) in the solution with constant pH control up to 2.0. The growth of oxidizing properties is also due to the presence of Fe(III) ions in the solution. The highest output of zinc dissolved from sulphide concentrates can be obtained in such conditions where low pH guarantees the stability of Fe(III) ions in the solution. At the same time there is no possibility to precipitate Fe(III) ions into the sediments. This has been confirmed by the previously presented data (Fowler et al. 1998).

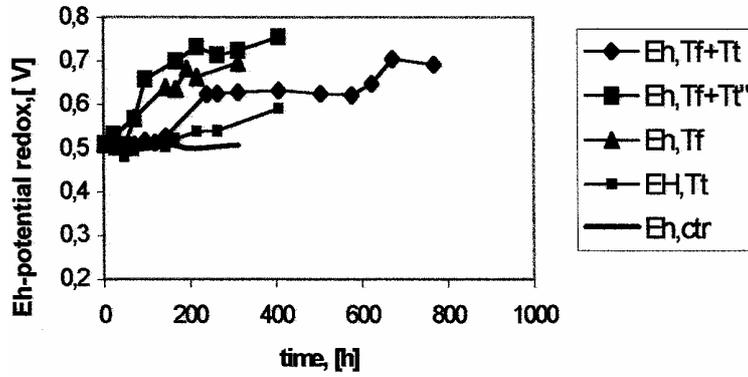


Fig. 4. Eh - redox potential during leaching of the blende concentrate in the presence of : *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* and mixed cultures. (")-adjusting to pH 2.0

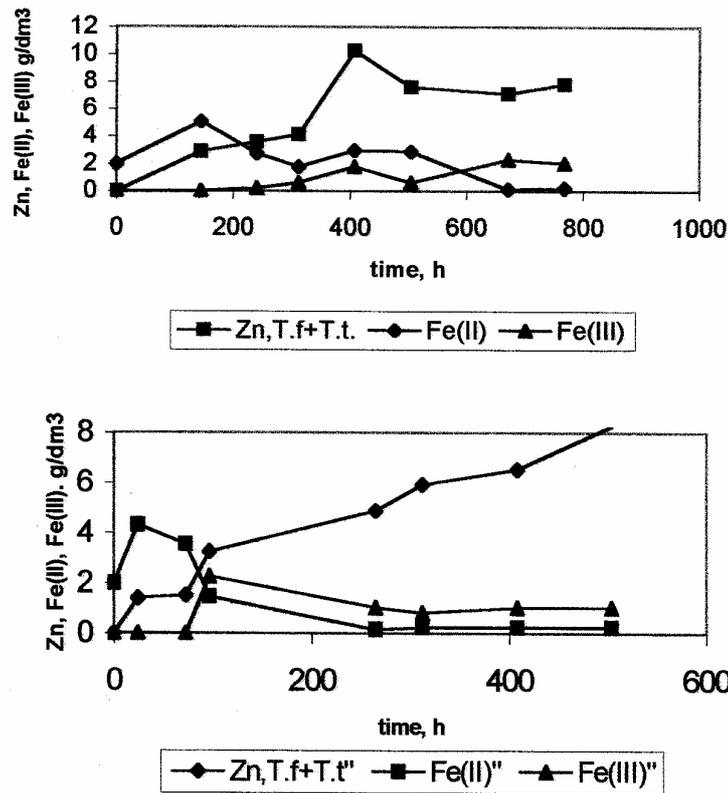


Fig.5. Changes in Zn, Fe(II) and Fe(III) concentration during bioleaching of blende flotation concentrate using mixed culture of *A. ferrooxidans* and *A. thiooxidans*, and (") with adjusting pH

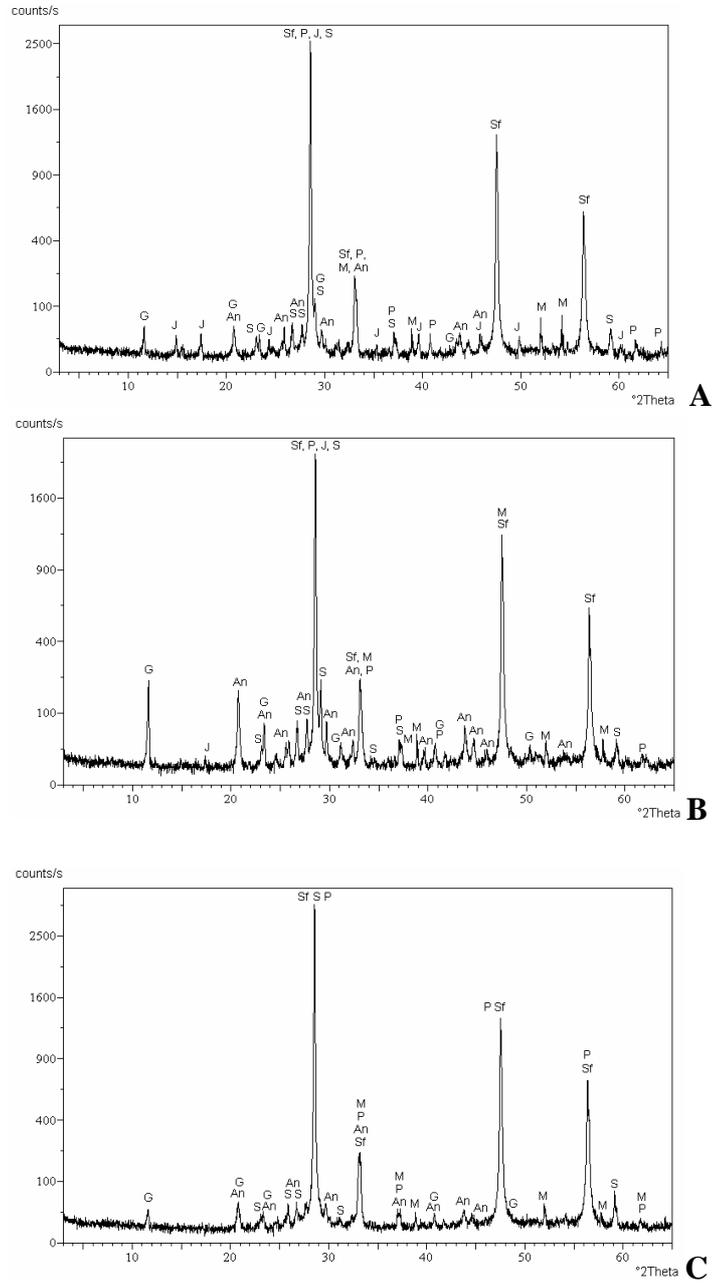


Fig. 6. X-ray diffraction patterns recorded for the products after A- bioleaching using *A. ferrooxidans*, B- bioleaching using the mixed cultures of *A. ferrooxidans* and *A. thiooxidans*. and C-control leaching of blende concentrate in 2K solutions: J-hydronium and potassium jarosite, Sf-sphalerite, S-sulphur, An-anglesite, G-gypsum, M-markasite, P-pyrite

EFFECT OF Fe(II) AND Fe(III) CONCENTRATION

The changes of Fe(II) and Fe(III) concentration in leaching medium using mixed cultures of *A. ferrooxidans* and *A. thiooxidans* have been presented in Fig.5. It is demonstrated that a rapid rate of dissolution of sphalerite from blende concentrate depends on a sufficient concentration of iron (III) ions in solution. Iron (III) ions are importance for cell attachment and degradation of metal sulphides, as described by (Sand et al. 2001). Correcting pH to low value provided a constant concentration of Fe(III) ions in solution (Fig. 5).

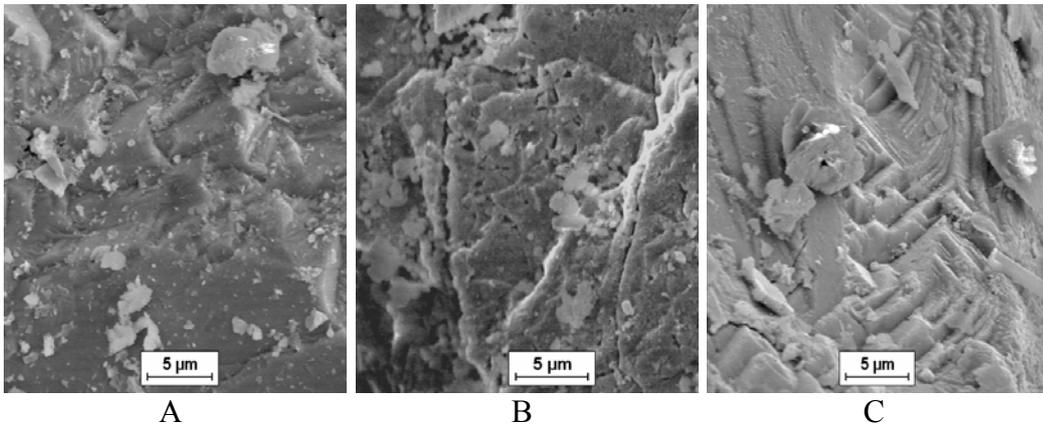


Fig. 7. Scanning electron microscopy of samples: A-blende (sphalerite) flotation concentrate, B-blende after bioleaching using *A. ferrooxidans*, C- sterile control leaching

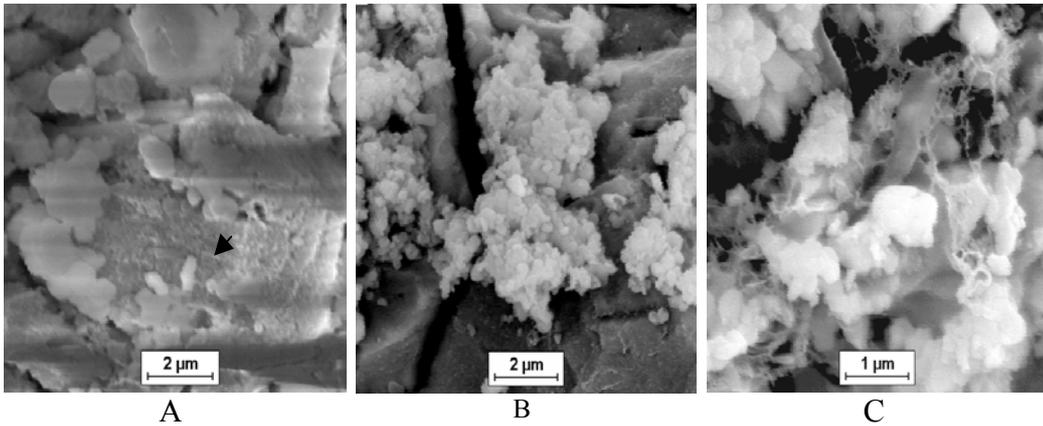


Fig. 8. Scanning electron microscopy image of blende (sphalerite) surface with cells (on image A indicated by arrow). B-complex of cells, C- extracellular polymeric substances (?) produced by *A. ferrooxidans*

#### X-RAY DIFFRACTION STUDIES

The X-ray diffraction patterns of the residues were examined for crystalline phases and it was observed that the new identifiable phases were jarosite, sulphur, anglesite and gypsum. It was stated that samples obtained after leaching using *A. ferrooxidans* consist of two new phases: the crystalline jarosite (hydronian jarosite) - about 8 % and sulphur-about 3 % - Fig.6A. In the samples after leaching using mixed cultures of *A. thiooxidans* and *A. ferrooxidans* there was a higher amount of elemental sulphur-about 4% (Fig. 6B). In some cases, samples without bacteria, no jarosite phase was identified, amount of elemental sulphur was about 2% (Fig. 6C)

#### SCANNING ELECTRON MICROSCOPY ANALYSIS

In order to determine the presence of minor components and bacteria in solid residues electron microscopy techniques were used. SEM revealed several important differences between solids precipitated from different solutions. Observations of the residue of concentrate by SEM showed it had undergone important changes compared with the biologically untreated concentrate. The angular forms of the particles had been rounded and smoothed (Fig. 7). The bacteria were present on the surface as the microcolonies or the single microorganism (Fig. 8). Possibly, there are extracellular polymeric substances excreted by bacteria *Acidithiobacillus ferrooxidans*.

#### CONCLUSIONS

The results obtained in this research work show that higher yields of extraction of zinc from blende (sphalerite) flotation concentrate can be obtained using a mixed culture of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* in comparison to a single culture of bacteria. One of the reasons of the low leaching capacity is the lack of oxidizing agent in leaching medium i.e. Fe(III) ions. The main cause of it is that the experimental conditions favoured jarosite compounds generation and precipitation. The layer of jarosites and sulphur, which were generated during the course of leaching time hindered bacterial action. It was observed that in the presence of different microorganism in the leaching medium there has been a succession in the treatment of components of sulphide minerals from flotation concentrate. At first reduced sulphur compounds appeared to be oxidized, and then ferrous ions. It was observed that the cultures of microorganisms from mineral water sources used in this study have been active in the experimental conditions and the increase of zinc and other metal leached from blende flotation concentrate were non toxic for them.

## ACKNOWLEDGEMENTS

The work was supported by the State Committee for Scientific Research in Poland, Project No.7 T09D 00221

## REFERENCES

- AHONEN L., TUOVINEN O. (1995), *Bacterial leaching of complex sulfide ore samples in bench-scale column reactors*, Hydrometallurgy Vol. 37, 1-21.
- BOON M., SNIJDER M., HANSFORD G.S., HEIJNEN J.J. (1998), *The oxidation of zinc sulphide with Thiobacillus ferrooxidans*, Hydrometallurgy Vol. 48, 171-186.
- CHENG-HSIEN HSU, HARRISON ROGER G. (1995), *Bacterial leaching of zinc and copper from mining wastes*. Hydrometallurgy Vol. 37, 169-179.
- CWALINA B. (1994), *Metabolizm siarki u Thiobacillus ferrooxidans w procesie ługowania metali z minerałów siarczkowych*. Wyd. UŚ, Katowice.
- CWALINA B., DZIERŻEWICZ Z., FARBISZEWSKA T., MIKLUSZKA K. (1994), *Zdolność biotransformacji nieorganicznych związków siarki przez wybrane szczepy autotroficznych i mikotroficznych bakterii siarczkowych i żelazowych*, Biotechnologia, 2 (25), 67-74.
- DAS R. P., ANAND S. (1996), *Thermogravimetric and XRD studies on iron compounds precipitated from Fe(II)-SO<sub>4</sub>-NH<sub>3</sub>-O<sub>2</sub> system*. Int. J. Miner. Process. Vol. 48, 159-168.
- FOWLER T.A., CRUNDWELL F.K. (1998), *Leaching of Zinc Sulfide by Thiobacillus ferrooxidans: Experiments with a Controlled Redox Potential Indicate No direct Bacterial Mechanism*. Applied and Environmental Microbiology, Vol.64, No 10, 3570-3575.
- FOWLER T.A., CRUNDWELL F.K. (1999), *Leaching of Zinc Sulfide by Thiobacillus ferrooxidans : Bacterial Oxidation of the Sulfur Product Layer Increases the Rate of Zinc Sulfide Dissolution at High Concentration of Ferrous Ions*, Applied and Environmental Microbiology, Vol.65, No 12, 5285-5292.
- GORMELY L.S., DUNCAN D.W., BRANION R.M.R., PINDER K.L. (1975), *Biotechnology and Bioengineering*, Vol.17, 31.
- HANSFORD G.S., VARGAS T. (2001), *Chemical and electrochemical basis of bioleaching process*, Hydrometallurgy, Vol. 59, 135-145.
- KARAVAIKO G.I. (1985), *Microbiological Processes for Leaching of Metals from Ores*, ed.Torma A.E., Moscow.
- KONISHI Y., KUBO H., ASAI S. (1992), *Bioleaching of zinc sulfide concentrate by Thiobacillus ferrooxidans*, Biotechnology and Bioengineering, Vol.39, No1, 66-74.
- LAZAROFF N., SIGAL W., WASSERMAN A. (1982), *Iron Oxidation and Precipitation of Ferric Hydroxysulfates by Resting Thiobacillus ferrooxidans Cells*, Applied and Environmental Microbiology, Vol. 43, No 4., 924-938.
- LIZAMA H. M., SUZUKI I. (1989), *Bacterial leaching of a sulfide Ore by Thiobacillus ferrooxidans and Thiobacillus thiooxidans. Part II. Column Leaching studies*. Hydrometallurgy Vol.. 22, 301-310
- LOCHMANN, PEDLIK M.(1995), *Kinetic anomalies of dissolution of sphalerite in ferric sulfate solution*. Hydrometallurgy, Vol. 37, 89-96.
- LOPEZ -DELGADO A., ALGUACIL F.J., LOPEZ F.A. (1997), *Recovery of iron from bio-oxidized sulphuric pickling waste water by precipitation as basic sulphates*, Hydrometallurgy Vol. 45, 97-112.
- ORŁOWSKA B., GOŁĄB Z.(1990), *Thiobacillus ferrooxidans - jako czynnik biologicznego ługowania metali*, Post. Mikrobiol., XXIX, No 3-4, 185-203.
- PACHOLEWSKI A., PACHOLEWSKA M. (2001), *Naturalne zdolności do utleniania związków żelaza (II) przez bakterie żelazowe ze źródła wody mineralnej Łomniczanka*. Współczesne Problemy Hydrogeologii, t.X, 389-396.
- PISTORIO M., CURUTCHET G., DONATI E., TEDESCO P. (1994), *Direct zinc sulphide bioleaching by Thiobacillus ferrooxidans and Thiobacillus thiooxidans*, Biotechnology Letters Vol. 16, No4, 419-424.

- SAND W., GEHRKE T., JOZSA P-G., SCHIPPERS A. (2001), *(Bio)chemistry of bacterial leaching - direct vs. indirect bioleaching*. Hydrometallurgy, 59, 159-175.
- SILVERMAN M.P., LUNDGREN D.G. (1959), J. Bacteriol., vol.77, , 642-647.
- SKŁODOWSKA A. (1990), *Utlnianie mineralnych związków siarki przez bakterie z rodzaju Thiobacillus*, Post. Microbiol., XXIX, No 1-2, 29-41.
- TORMA A.(1998), *Leaching of Metals*, Chapter 12 [in:] *Biotechnology - A Comprehensive Treatise in 8 Volumes*, ed. H.-J. Rehm, G.Reed, Vol.6 b, Weinheim.

**Pacholewska M.**, Mikrobiologiczne ługowanie koncentratów blendy flotacyjnej przy użyciu *Acidithiobacillus ferrooxidans* oraz *Acidithiobacillus thiooxidans*, Physicochemical Problems of Mineral Processing, 37 (2003) 57-68 (w jęz. ang.).

W pracy przedstawiono wyniki badań nad mikrobiologicznym ługowaniem krajowych siarczkowych koncentratów blendy flotacyjnej pochodzących z ZGH "Bolesław" przy użyciu szczepów bakterii *Acidithiobacillus ferrooxidans* oraz *Acidithiobacillus thiooxidans* w roztworach 2K stosowanych jako pożywki dla wzrostu bakterii żelazowych. Stopień rozтворzenia cynku z koncentratu blendowego do roztworu wyniósł 40,28 % dla prób biologicznych z udziałem mieszanych kultur bakteryjnych oraz 22,69 % dla prób prowadzonych w warunkach sterylnych. Stwierdzono, że reakcja rozтворzenia cynku ulega zahamowaniu przez powstające stałe produkty reakcji jarosyty i siarkę elementarną. Prowadzenie ługowania koncentratu blendowego przy stałej korekcie pH<sub>2,0</sub> roztworu umożliwia częściowe zachowanie związków żelaza w roztworze, przeciwdziałając w ten sposób osadzaniu się stałych produktów reakcji. W toku badań zaobserwowano, że stosowane mieszane kultury bakteryjne wykazują sukcesję w odniesieniu do procesu rozтворzenia minerałów siarczkowych utleniając początkowo jony siarczkowe a następnie jony żelaza (II) a ponadto są odporne na obecność jonów cynku w roztworze.