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BEATA CWALINA*, MAŁGORZATA PACHOLEWSKA**,
MARIA SOZAŃSKA***, JERZY CABAŁA****

MICROENVIRONMENTS DETERMINING GROWTH OF ACIDOPHILIC BACTERIA IN Zn-Pb FLOTATION TAILINGS OF NEUTRAL REACTION

Zinc and lead ore flotation tailings deposited in the Olkusz region show a neutral reaction. They are composed of dolomite-type minerals (alkaline), mostly $Ca,Mg(CO_3)_2$ and $Ca,Mg,Fe(CO_3)_2$ (70%), and other minerals including sulphides (acid), mainly pyrite and marcasite (FeS_2; up to 20%). Sulphur and iron concentration in tailings reaches the levels of 7–14% and of 8–16%, respectively. The microenvironments rich in sulphide minerals present in waste materials which indicate almost neutral reaction allow the metabolic activity of acidophilic sulphur- and iron-oxidizing bacteria of *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* species to be grown and developed, in spite of the alkaline character of basic mineral components in the tailings examined.

1. INTRODUCTION

Acidophilic sulphur- and iron-oxidizing bacteria are commonly present in the environments rich in sulphide minerals, primarily those which contain pyrite and marcasite (FeS_2). The sulphide oxidation processes (both abiotic and biotic) lead to the environment acidification and the mobilization of metals due to the leaching processes. Such environments are suitable for the growth and metabolic activities of acidophilic sulphur- and iron-oxidizing bacteria [1]–[4].

Zinc and lead post-flotation tailings which are deposited in the Olkusz region, southern Poland, contain almost 70% of dolomite minerals such as $Ca,Mg(CO_3)_2$ and $Ca,Mg,Fe(CO_3)_2$. Sulphide minerals, mainly pyrite and marcasite (FeS_2, 15–20%), are predominant constituents among other minerals [5]. The sulphur and iron concentra-

* Department of Environmental Biotechnology, Silesian University of Technology, ul. Akademicka 2, 44-100 Gliwice, Poland. E-mail: Beata.Cwalina@polsl.pl;

** Department of Metallurgy, Silesian University of Technology, Katowice, Poland.

*** Department of Materials Engineering, Silesian University of Technology, Katowice, Poland.

**** Faculty of Earth Sciences, University of Silesia, Sosnowiec, Poland.

tion in flotation tailings may reach 7–14% and of 8–16%, respectively. In sedimentary ponds, the complexity of waste materials deposited in different places and layers leads to the occurrence of various extremophile bacteria, including acidophilic bacteria capable of sulphur- and iron-oxidation [6]. Some of these bacterial strains tolerate high concentrations of metal ions [7] and flotation reagents [8].

The aim of this paper was to study specific microenvironments found in Zn-Pb flotation tailings, whose pH was almost neutral, with reference to the potential presence and activity of acidophilic bacteria, especially *Acidithiobacillus* genus, which are able to oxidize inorganic sulphur and iron compounds.

2. MATERIALS AND METHODS

The post-flotation waste samples were collected from the Bolesław-Olkusz tailings impoundments (figure 1), in which the waste materials produced during Zn-Pb ores treatment are deposited. The analyses were carried out in order to search and characterize the sulphur- and sulphide-rich microenvironments suitable for the growth and metabolic activities of acidophilic bacteria able to oxidize the reduced sulphur- or/and iron-bearing mineral substrates.

Fig. 1. Storage yard of post-flotation tailings produced during processing Zn-Pb ores in ZGH Bolesław SA (A); the tailings pond (B)

The mineral composition of post-flotation tailings was analyzed by scanning electron microscopy (SEM) with energy dispersive X-ray spectrometry (EDS). SEM-EDS analyses were carried out using SEM Hitachi S-4200 equipped with EDS Voyager 3500 system.

Acidophilic bacteria were isolated from solid post-flotation tailings and from water and foam occurring on the surface of sedimentary ponds (figure 1) as well as from water effluents from these sedimentary pond slopes (figure 2).

Fig. 2. Sulphates crystallized from acid drainages (back-slope of tailings impoundment)

The samples of waste materials and their water extracts were introduced into the sterile liquid culture media containing two types of substrates used as sole energy sources for the bacteria tested. These substrates included sulphur or thiosulphate stimulating the growth of sulphur-oxidizing bacteria [9], or Fe(II)-ions stimulating the growth of iron-oxidizing bacteria [10].

The composition of a liquid medium of WAKSMAN and JOFFE [9] was as follows (g/dm^3): $(NH_4)_2SO_4$, 0.2; KH_2PO_4, 3.0; $MgSO_4{\cdot}7H_2O$, 0.5; $CaCl_2{\cdot}6H_2O$, 0.25; $FeSO_4{\cdot}7H_2O$, traces; S^o, 10.0 (or $Na_2S_2O_3$, 20.0); pH 4.0 (addition of 10 N H_2SO_4). The liquid medium 9K of SILVERMAN and LUNDGREN [10] contained (g/dm^3): $(NH_4)_2SO_4$, 3.0; KCl, 0.1; K_2HPO_4, 0.5; $MgSO_4{\cdot}7H_2O$, 0.5; $Ca(NO_3)_2$, 0.01; $FeSO_4{\cdot}7H_2O$, 44.2 (9 g Fe^{2+} in 1 dm^3 of the medium); pH 2.5 (addition of 10 N H_2SO_4). Bacteria were grown in liquid media at 30 °C for 3–5 days. Then, the liquid samples were successively diluted in the same liquid media and plated (in triplicate) on the same types of solid media adequate for the searched acidophilic bacteria. Separate colonies were selected and inoculated into respective liquid media. Cultures were purified by a repeated plating and a single colony isolation. The isolated bacteria were identified on the basis of 16S rRNA analyses.

3. RESULTS AND DISCUSSION

As is widely known, chemolithotrophic acidophilic sulphur- and iron-oxidizing bacteria oxidize sulphide minerals such as pyrite, marcasite, sphalerite, wurzite, etc. *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* are the most common iron-oxidizing bacteria usually accompanied by sulphur-oxidizing acidophiles, e.g. *Acidithiobacillus thiooxidans,* which can oxidize sulphur and inorganic sulphur compounds, such as thiosulphate, formed during sulphide minerals oxidation.

Some strains of acidophilic sulphur- and iron-oxidizing bacteria, mostly *A. ferrooxidans* and *A. thiooxidans*, have been found in the post-flotation tailings. Some strains representing the above-mentioned species (degree of identity based on 16S rRNA), isolated from the selected waste samples, are described in the table, which contains also the bacteria origin description and pH-values of solid, foam and water samples. It is worth denoting a near neutral reaction of the tailings (pH 6.50–7.27).

Table

Some selected acidophilic sulphur- and iron-oxidizing bacteria isolated from post-flotation tailings (degree of identity based on 16S rRNA)

Strain symbol	Origin description	pH	Genus and species	Identity (%)
SB1	Deposit from drainpipe; the oldest part of sedimentary pond (SP)	7.10	*Acidithiobacillus ferrooxidans*	98
WA1.3	Solid waste (SW) from dump; the oldest part of SP; depth of 3 m	6.50	uncultured bacteria // *Thiobacillus* sp.	93
WA2.0	SW from dump; the youngest part of SP; surface	7.10	uncultured bacteria // *Thiobacillus plumbophilus*	97/99
WA6.2004	Infiltrating water effluent from SP toe	7.22	*Acidithiobacillus thiooxidan*s	99
WA11	Water from surface of medium-age part of SP	7.27	*Acidithiobacillus ferrooxidans*	99
WA11.0	SW from surface of medium-age part of SP	7.17	*Thiobacillus neapolitanus* // *Halothiobacillus neapolitanus*	99
WA11P	Foam from surface of medium-age part of SP	7.20	*Acidithiobacillus thiooxidan*s	99

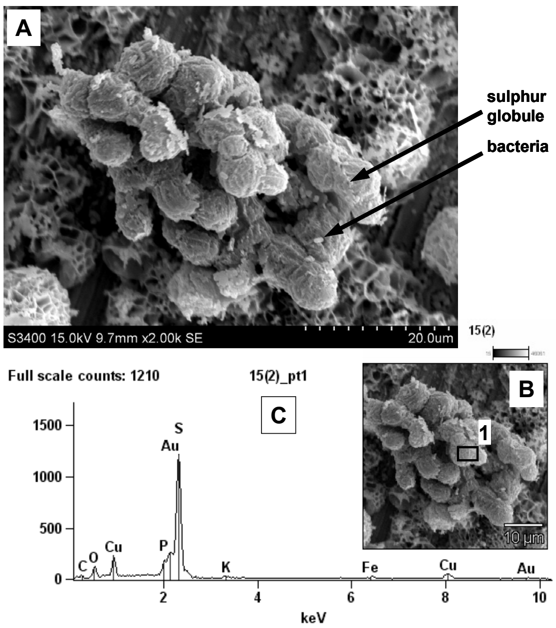

Fig. 3. Sulphur-oxidizing bacteria settled on sulphur globules in culture medium

The isolation of a large number of *A. ferrooxidans* and *A. thiooxidans* strains from the Zn-Pb flotation tailings testifies to favourable conditions for growth and high metabolic activity of these bacteria, being proved in our previous studies [6]–[8]. The presence of acidophilic sulphur-oxidizing bacteria in a liquid medium containing sulphur as the sole energy source for the bacteria growth was well documented by SEM (figure 3).

The results obtained using the SEM-EDS microanalysis method confirmed our earlier findings [5] that post-flotation waste materials contained mainly carbonate minerals of the dolomite type (figures 4 and 5). Sulphide and silicate minerals (including aluminosilicates) as well as oxide minerals are also present in these wastes. Sulphide assemblages are dominated by pyrite (FeS_2; figures 5 and 6) and sphalerite (ZnS; figures 4 and 7).

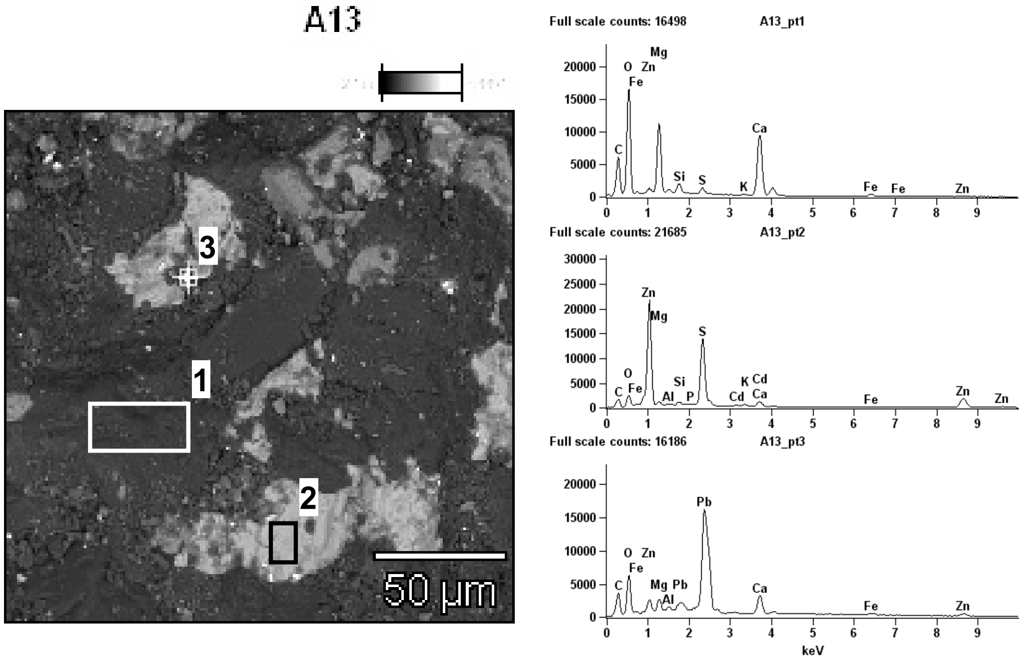

Fig. 4. Post-flotation tailings rich in dolomite-type minerals with inclusions of sulphide and other minerals

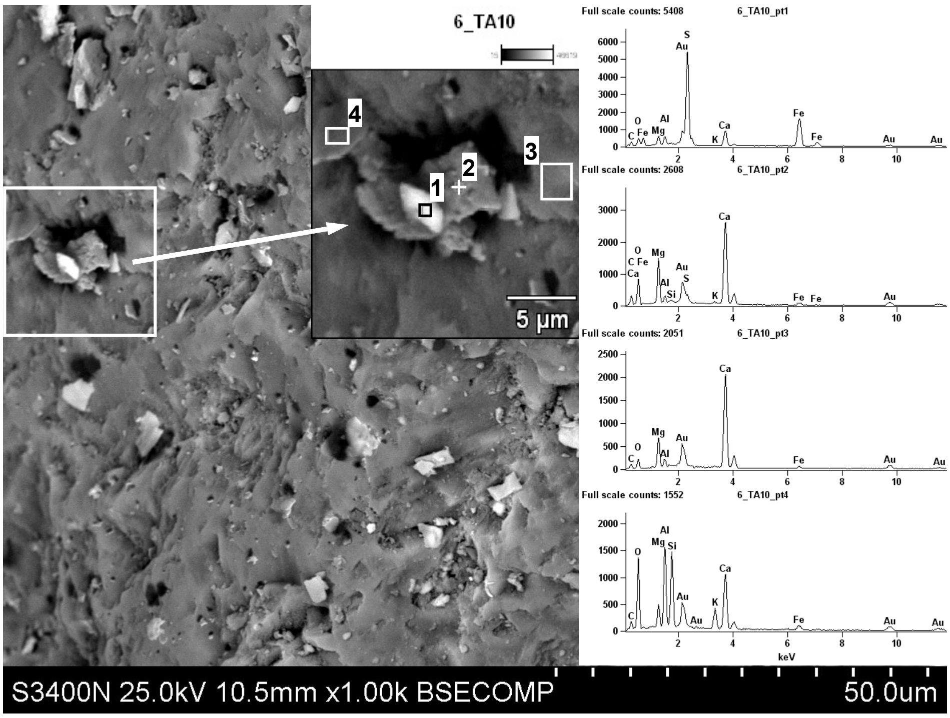

Fig. 5. Iron sulphide in matrix of various calcium-rich minerals

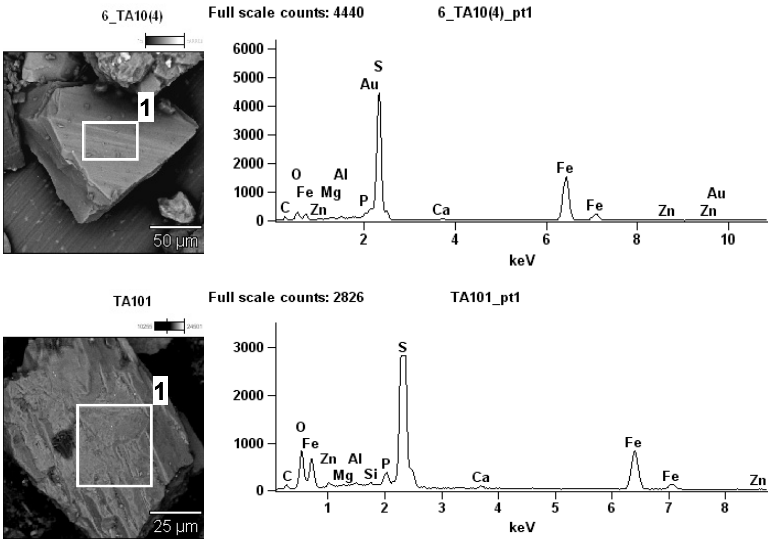

Fig. 6. Pyrite grains: showing no sign of weathering (head)
and indicating effects of this process (bottom)

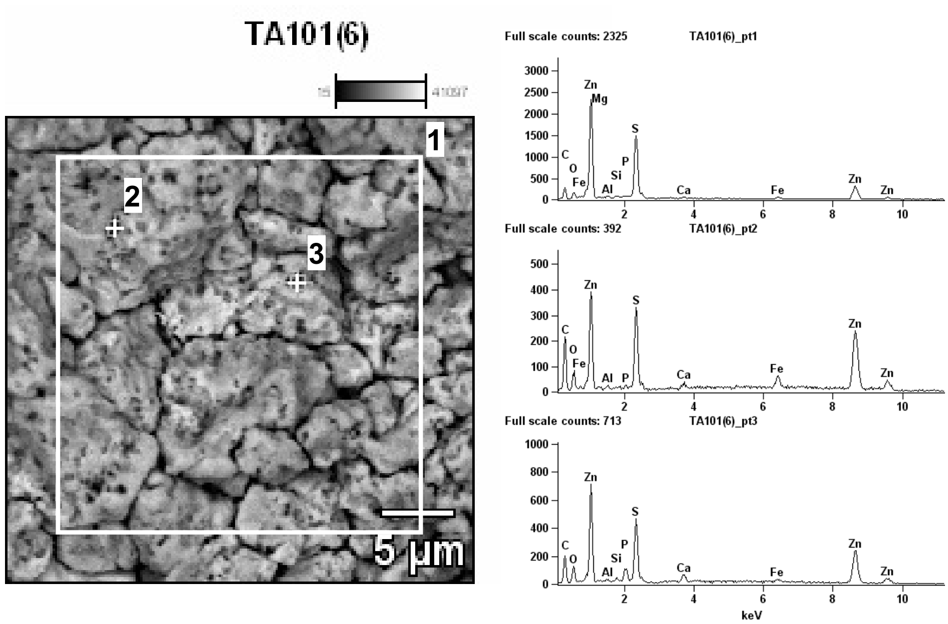

Fig. 7. Zinc sulphide mineral (probably sphalerite, ZnS) of porous structure

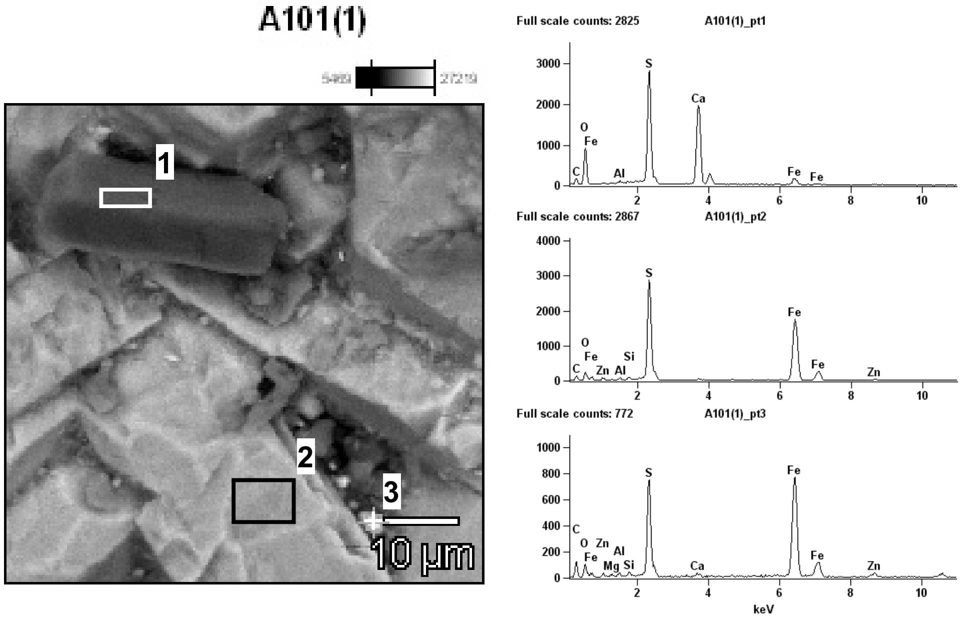

Fig. 8. Pyrite grains with secondary simple and complex sulphate-type minerals, dominated by gypsum

Many pyrite grains show no sign of weathering (figure 6, head), whereas on some of them the effects of this process are visible (figure 6, bottom; figure 8). The oxidation of sulphide minerals (both abiotic and biotic) leads to the production of sulphuric acid, which reacts with carbonate and other minerals to form simple and complex sulphate-type minerals. These secondary sulphate minerals are dominated by gypsum (figure 8), although various jarosite-type minerals were also detected in some samples. An influence of the infiltrating water on mineral composition is clearly manifested in the lower parts of the sedimentary pond slopes (figure 2).

4. CONCLUSIONS

1. The sulphide, sulphate and other mineral compounds of various metals (mainly of Fe, Zn, Pb) form micro-aggregates in the Zn-Pb post-flotation tailings under examination.

2. Various sulphide minerals, mostly pyrite and marcasite (FeS_2) as well as the sphalerite (ZnS), in the waste materials under examination stimulate the growth and metabolic activity of acidophilic sulphur- and iron-oxidizing bacteria, including those of *Acidithiobacillus* genus. Bacterial strains belonging to *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* species were identified on the basis of the 16S rRNA analyses.

3. The presence of microenvironments rich in sulphide minerals in the examined Zn-Pb post-flotation tailings of generally near neutral reaction allowed the development and metabolic activity of acidophilic sulphur- and iron-oxidizing bacteria of *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* species, in spite of the alkaline character of the basic mineral components in the waste material.

4. The secondary sulphate minerals found in the Zn-Pb post-flotation tailings examined can be of both abiotic and biotic origins.

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MIKROŚRODOWISKA UMOŻLIWIAJĄCE ROZWÓJ BAKTERII KWASOLUBNYCH W ODPADACH POFLOTACYJNYCH RUD CYNKU I OŁOWIU O OBOJĘTNYM ODCZYNIE

Odpady poflotacyjne rud cynku i ołowiu zdeponowane w okolicach Olkusza wykazują odczyn obojętny. Zawierają one minerały typu dolomitu (alkaliczne), głównie $Ca,Mg(CO_3)_2$ i $Ca,Mg,Fe(CO_3)_2$ (70%), oraz inne minerały, włączając siarczki (kwaśne), głównie piryt i markasyt (FeS_2; do 20%). Stężenia siarki i żelaza w odpadach osiągają poziomy odpowiednio 7–14% oraz 8–16%. Pokazano, że obecność mikrośrodowisk bogatych w minerały siarczkowe w materiałach odpadowych wykazujących generalnie odczyn obojętny umożliwia rozwój i aktywność metaboliczną kwasolubnych bakterii utleniajacych siarkę i żelazo, a należących do gatunków *Acidithiobacillus thiooxidans* i *Acidithiobacillus ferrooxidans* pomimo alkalicznego charakteru podstawowych składników mineralnych w badanych odpadach.