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THE INFLUENCE OF FLOTATION REAGENTS ON SULFUR-OXIDIZING BACTERIA *ACIDITHIOBACILLUS THIOOXIDANS*

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It has been demonstrated that various flotation reagents influence, in a different manner, the metabolic activity of the sulfur-oxidizing bacteria *Acidithiobacillus thiooxidans* (strain C1 being isolated from the Fe-Zn tailings water) growing in the Waksman/Joffe (W/J) liquid culture medium that contains thiosulfate as a sole energy source for bacteria growth. The ethyl- and amyl xanthates as well as the frothing reagent stimulated, to a limited extent, the tested C1-bacteria metabolic activity. The very well documented bacterially-influenced acidification of the W/J solution supplemented with the ethyl- or the amyl xanthates suggests the possibility of these substances effective acid-degradation in the post-industrial environments rich in various flotation reagents, mainly xanthates. Both the activator containing the carbamate ethyl-derivative and the modifier composed of Cu(II)-ions caused a complete inactivation of the *A. thiooxidans* C1-metabolism. It is suggested that some unexpected chemical reactions may proceed in the tested systems, as a result of interactions between the culture medium components, flotation reagents, their decomposition products, and also the products of bacterial metabolism.

key words: Acidithiobacillus thiooxidans, metabolic activity, flotation reagents, xanthates, frother, activator, Cu(II)-ions

INTRODUCTION

Flotation is a method used for enrichment of millions tons (Tg, teragrams) of mineral raw materials, including 80-90% of non-ferrous metal ores mined in the world. In

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Poland, nearly 30 Tg of copper ores as well as about 5 Tg of zinc-lead ores are concentrated by this method. In the minerals processing, flotation is one of the most important physicochemical methods of the mineral raw materials enrichment (Drzymała, 2007; Laskowski and Łuszczkiewicz, 1989). This method is also of high efficiency in the zinc and lead sulfide minerals selective separation from crude ores. No chemical changes in the ore mineral components take place during flotation processes. Initial stage of the ore enrichment in the heavy media with either ferrosilicon or magnetite makes possible the barren dolomite separation from the heavier aggregates. The dolomite gangues are widely used as the aggregate in building materials. The pre-enriched ore is milled, and then floated, using many flotation reagents including:

- collector reagents - mainly xanthates (ethyl, butyl, amyl), alkyl sulfates, and aliphatic amine hydrochlorides
- frothing reagents - octanol and diacetone alcohol mixtures with the mesityl oxide
- modifying reagents - activators, depressors, pH regulators.

A number of flotation reagents remain in the post-flotation waste materials, which are composed mainly of the carbonate gangues (70%), Fe sulfides (pyrite and marcasite, 15-20%), non-floatable small fractions of the Pb and Zn sulfide minerals, as well as Zn, Pb, Cd, As and Tl oxide minerals. The post-flotation tailings resulting from processing of the Zn-Pb ores originated from the Pomorzany, Olkusz and Bolesław mines are stored in the Olkusz region. Here, the post-mining grounds and storage yards (containing both present and historic waste materials) occupy an area of about 2000 ha.

Chemical stability of the flotation reagents such as xanthates depends mostly on pH of the environment. Significant increase in acidity (pH 3-5) leads to xanthates degradation according to overall reaction: $ROCS_2H \rightarrow ROH + CS_2$ (Zhongxi and Forsling, 1999).

The sulfide minerals present in the post-flotation waste materials form a habitat favorable to the acidophilic sulfur bacteria growth (Cwalina and Jaworska-Kik, 2008; Pacholewska et al., 2007). Their ability to oxidize sulfur compounds leads to environment acidification. Thus, the xanthates decomposition may result from the sulfur-oxidizing bacteria metabolic activity, although the biological degradation of flotation reagents is rather attributed to the bacterium *Bacillus polymyxa* action (Deo and Natarajan, 1998). On the other hand, it seemed to be probable that xanthates could be used as sulfur-bearing substrates for the sulfur-oxidizing bacteria growth due to chemical composition of these substances group (Fig. 1). Although it has been demonstrated by Hoon and Madgwick (1995) that ethyl, amyl and isobutylxanthates cause inhibition of the *Acidithiobacillus ferrooxidans* bacteria growth, but we observed a high metabolic activities of the *A. ferrooxidans* and *Acidithiobacillus thiooxidans* strains isolated from the flotation tailings rich in various flotation reagents including xanthates (Pacholewska et al., 2007; Cwalina and Jaworska-Kik, 2008). This finding pointed to

these bacteria adaptation to such environment and suggested possibility of xanthates use for their growth.

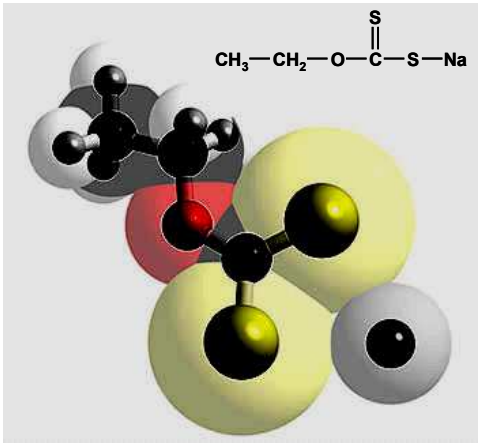

Fig. 1. Sodium ethyl xanthate structure (after http://www.3dchem.com; with modification)

The aim of present work was to study the influence of various flotation reagents on the metabolic activity of the sulfur-oxidizing bacteria *Acidithiobacillus thiooxidans* being isolated from the Fe-Zn tailings water. The main question was, whether or not the tested bacteria metabolic activity is stimulated or depressed by the flotation reagents. On the other hand, it seems to be probable that the biogenic sulfuric acid resulted from the high metabolic activity of the *A. thiooxidans* bacteria may cause the flotation reagents degradation. Both the flotation reagents as well as their decomposition products appear to have effect on the bacteria growth and activity. This activity has been evaluated basing on acidifying the Waksman/Joffe (W/J) liquid culture medium (Waksman and Joffe, 1922) that contains thiosulfate as the sole energy source for bacterial growth.

MATERIALS AND METHODS

Investigations have been carried out using C1 strain of *Acidithiobacillus thiooxidans* bacteria (Fig. 2) of high metabolic activity, being able to oxidize sulfur and its inorganic compounds (Cwalina and Jaworska-Kik, 2008; Pacholewska et al., 2007). This bacteria strain has been isolated from the acid water sampled from the piezometer P1 installed in the Fe-Zn tailings pond (HMN Szopienice, Katowice). Bacteria were cultured in the W/J liquid medium (Waksman and Joffe, 1922) containing (g/dm^3): $Na_2S_2O_3 \cdot 5H_2O$ - 5.0; KH_2PO_4 - 3.0; $MgCl_2 \cdot 6H_2O$ - 0.1; $CaCl_2 \cdot 6H_2O$ - 0.25; NH_4Cl - 0.1; $FeSO_4 \cdot 7H_2O$ - traces; pH 4.0. The culture medium was supplemented with the flotation reagents being used for the Zn-Pb ores enrichment during their in-

dustrial processing in the Olkusz mine. The reagents have been obtained from the Ores Enrichment Plant ZGH Bolesław S.A. Additives were introduced into the W/J liquid medium to provide concentrations proportional to those occurring under industrial conditions, taking into account the pulp density used in the experiments, i.e. 5 g of waste material in 100 cm^3 of the solution. Additions of the flotation reagents were as follows:

1. collector, amyl xanthate - 0.4 cm^3/100 cm^3 of W/J solution
2. collector, ethyl xanthate - 0.2 cm^3/100 cm^3 of W/J solution
3. frothing reagent, Corflot - 0.04 cm^3/100 cm^3 of W/J solution
4. activator/collector, Selkol 1981 - 0.01 cm^3/100 cm^3 of W/J solution
5. modifying reagent, the industrial solution $CuSO_4$ - 0.32 cm^3/100 cm^3 of W/J solution.

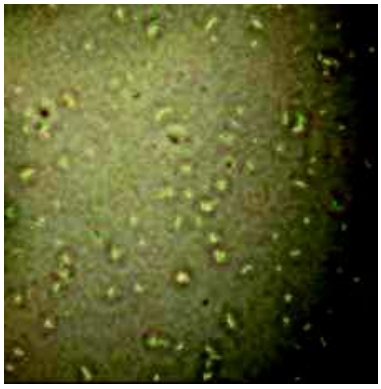

Fig. 2. Cells of *Acidithiobacillus thiooxidans* bacteria (strain C1) in the Waksman/Joffe (W/J) liquid culture medium. Optical microscope; picture taken at magnification 1000×

The liquid culture of the *A. thiooxidans* C1 bacteria strain growing in the W/J solution without flotation reagents as well as the sterile W/J media without or with flotation reagents have been used as the control systems. Bacteria were introduced as 2cm^3 aliquots of inoculum containing *A. thiooxidans* C1 population in the exponential phase of growth. Experiments were carried out at temperature of 20^oC, under mechanical shaking. The suspensions' pH has been measured using a combined glass electrode, type ERH 111 (Hydromet), and Slandi SP300 electronic pH-meter.

RESULTS AND DISCUSSION

A. THIOOXIDANS ACTIVITY IN THE PRESENCE OF XANTHATES

The results presented in Fig. 3 reflect the xanthates influence on *A. thiooxidans* (strain C1) metabolic activity. Only inconsiderable differences are visible between C1

cultures grown in W/J solutions without and with xanthates. The lowest and very similar values of pH (1.7-2.2) have been measured in all solutions inoculated with C1 bacteria after their culturing for 96 hours and longer (up to 192 hours). Besides, some beneficial effect of xanthates on the bacteria activity has been observed after 72 hours of the C1-strain growth in the W/J liquid medium supplemented with these flotation reagents, where the solution acidification was more intensive as compared with the xanthate-free bacterial culture. These results suggest that the *A. thiooxidans* bacteria metabolic activity was influenced by xanthates only to a small extent and that their presence was not disadvantageous for bacterial populations being used in the experiment. As a result, considerable acidification of the W/J solution occurred in all liquids inoculated with the *A. thiooxidans* C1 strain, leading to form favorable conditions for the xanthates degradation. Sterile W/J solution supplemented with the ethyl xanthate indicates no changes in its acidity throughout the experiment duration whereas some inconsiderable pH-increase has been observed in the W/J solution containing the amyl xanthate.

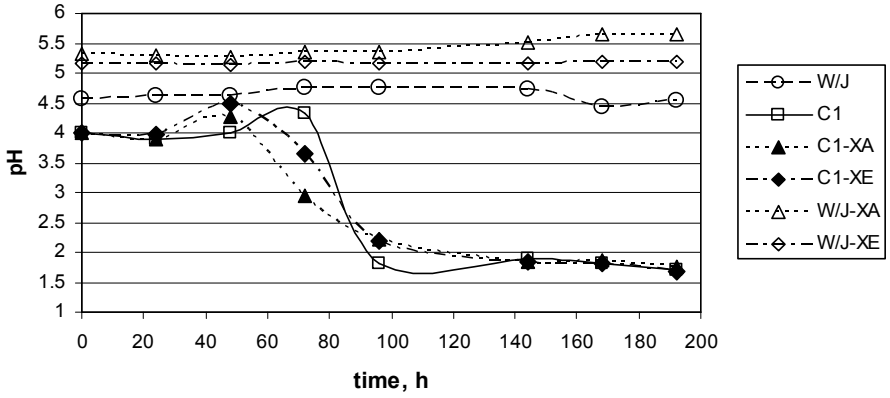

Fig. 3. The influence of xanthates on *A. thiooxidans* (strain C1) metabolic activity; W/J - sterile Waksman/Joffe liquid culture medium; C1 - W/J+C1 strain; XA - amyl xanthate; XE - ethyl xanthate

A. THIOOXIDANS ACTIVITY IN THE PRESENCE OF FROTHING REAGENT

The influence of the Corflot frothing reagent on metabolic activity of the *A. thiooxidans* bacteria (strain C1) has been shown in Fig. 4. It is worth to denote that dynamic curves indicating the W/J liquid culture medium acidification by C1 bacteria growing without and with the frothing reagent tested were almost identical. The Corflot frothing reagent did not cause the pH-changes in the W/J sterile solution.

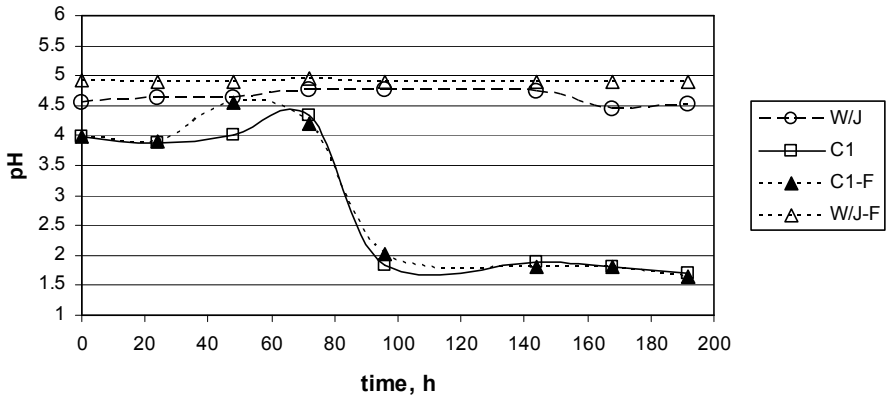

Fig. 4. The influence of frother on *A. thiooxidans* (strain C1) metabolic activity; W/J - sterile Waksman/Joffe liquid culture medium; C1 - W/J+C1 strain; F - frother

A. THIOOXIDANS ACTIVITY IN THE PRESENCE OF ACTIVATOR

Figure 5 summarizes results concerning the Selkol activator influence on the *A. thiooxidans* bacteria (strain C1) metabolic activity. Unfavorable effect of this flotation reagent is very essential and clear. No acidification took place in the C1-bacteria culture growing in the W/J liquid medium supplemented with Selkol. This flotation agent caused the bacteria metabolism inactivation from the beginning of bacteria culturing. The toxic effect of the Selkol activator is probably due to the presence in it of very toxic carbamate ethyl-derivative. The Selkol activator had no effect on pH of the W/J sterile solution.

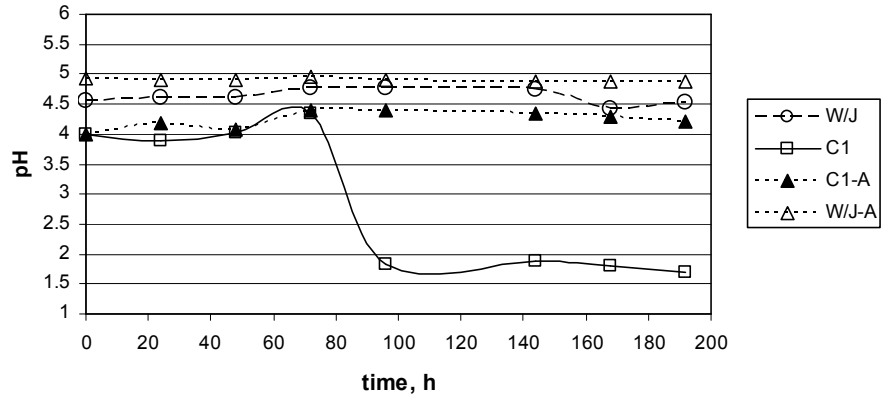

Fig. 5. The influence of activator on *A. thiooxidans* (strain C1) metabolic activity; W/J - sterile Waksman/Joffe liquid culture medium; C1 - W/J+C1 strain; A - activator

A. THIOOXIDANS ACTIVITY IN THE PRESENCE OF MODIFIER - Cu(II)

The results presented in Fig. 6 show the influence of Cu(II)-ions (being used in flotation processes as the modifying reagent) on the pH-changes in the W/J liquid culture media - both sterile and inoculated with *A. thiooxidans* bacteria (strain C1). No acidification has been denoted in the bacterial solution supplemented with copper ions. On the contrary - pH-changes towards the solution alkalization have been observed. This points to a highly toxic effect of these ions on the investigated bacteria strain. The *A. thiooxidans* bacteria sensibility to various metal ions, including Cu(II), will be presented in the separate paper.

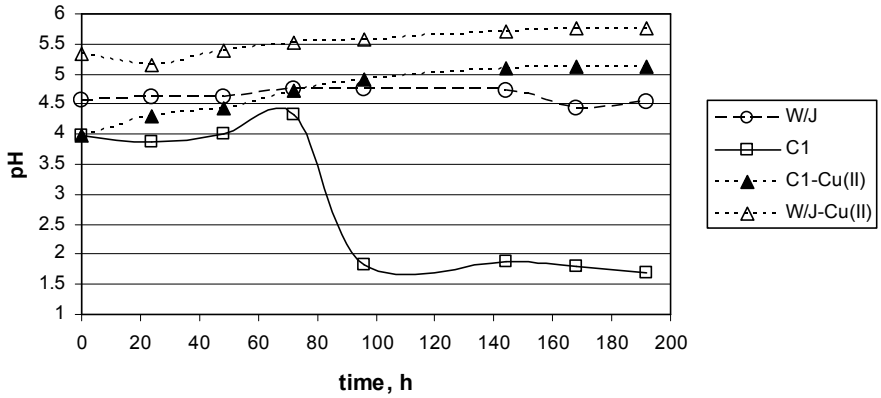

Fig. 6. The influence of Cu(II)-ions on *A. thiooxidans* (strain C1) metabolic activity; W/J - sterile Waksman/Joffe liquid culture medium; C1 - W/J+C1 strain; Cu(II) - $CuSO_4$

Apart from the *A. thiooxidans* C1-metabolism complete inactivation, an increase in the pH values being observed during the process run under sterile conditions suggests possibility of the thiosulfate destabilization due to the Cu(II)-ions chemical reaction (Senanayake, 2005).

THE OVERALL ANALYSIS OF FLOTATION REAGENTS INFLUENCE ON THE *A. THIOOXIDANS* METABOLIC ACTIVITY

The mean values of the rates of H^+-concentration changes in pure W/J liquid medium as well as in the media supplemented with selected flotation reagents, both sterile and inoculated with *A. thiooxidans* bacteria (strain C1), have been presented in Table 1. It may be stated that the bacteria presence in the W/J solution leads to about 10000-fold increase in the protons concentration - from about $2.0 \cdot 10^{-3}$ mmol/dm^3 up to 20.3±2.3 mmol/dm^3. Calculated values of the mean rates of H^+-production due to the bacteria activity were 2.52±0.25 mmol/dm^3/d, indicating the variability coefficient not exceeding 10%. All flotation reagents tested did cause some decrease in the H^+ con-

centration in the W/J solution. This effect seems to be more significant in solutions supplemented with the activator and the Cu(II)-ions, especially in the presence of bacteria. In this experimental system, the rate of H^+ concentration decrease was about 10-fold higher as compared with a parallel sterile solution. These results may point to both toxic effects of the activator and modifier on the bacteria metabolic activity as well as to chemical reactivity modification of these substances by the bacteria metabolites or the cells' lysis products liberated into the solution. On the other hand, it has been demonstrated for the first time that the ethyl and amylxanthates as well as the frothing reagent studied in this work may stimulate, to a limited extent, the metabolic activity of the bacteria *Acidithiobacillus thiooxidans* (Fig.3, 4; Tab. 1), but their role in the activation effect has not been studied as yet.

Table 1. Changes in H^+ concentration in pure Waksman/Joffe (W/J) liquid medium and in the media (sterile and inoculated with *A. thiooxidans* strain C1) containing flotation agents

Sample symbol	Addition of C1-bacteria and flotation agents (FA) to W/J solution	H^+ concentration increase (+) or decrease (-) [mmol/dm^3]	Rate of change in H^+-concentration [mmol/dm^3/d]
W/J	W/J sterile, without FA	$+2.0\cdot10^{-3}$	$+2.5\cdot10^{-4}$
C1	C1 without FA	+20.3	+2.5
C1-XA	C1 + amyl xanthate	+17.3	+2.2
C1-XE	C1 + ethyl xanthate	+20.8	+2.6
C1-F	C1 + frother	+22.8	+2.8
C1-A	C1 + activator	$-4.2\cdot10^{-2}$	$-5.3\cdot10^{-3}$
C1-Cu(II)	C1 + $CuSO_4$	$-9.5\cdot10^{-2}$	$-1.2\cdot10^{-2}$
W/J-XA	W/J + amyl xanthate	$-2.5\cdot10^{-3}$	$-3.2\cdot10^{-4}$
W/J-XE	W/J + ethyl xanthate	$-4.5\cdot10^{-4}$	$-5.6\cdot10^{-5}$
W/J-F	W/J + frother	$+5.5\cdot10^{-4}$	$+6.9\cdot10^{-5}$
W/J-A	W/J + activator	$+1.2\cdot10^{-3}$	$+1.5\cdot10^{-4}$
W/J-Cu(II)	W/J + $CuSO_4$	$-1.5\cdot10^{-2}$	$-1.8\cdot10^{-3}$

CONCLUSIONS

1. Various flotation reagents influence, in a different manner, the metabolic activity of the sulfur-oxidizing bacteria *Acidithiobacillus thiooxidans* (strain C1 being isolated from the Fe-Zn tailings water) growing in the Waksman/Joffe (W/J) liquid culture medium that contains thiosulfate as a sole energy source for bacteria growth.
2. It has been demonstrated that the ethyl- and amyl-xanthates as well as the frothing reagent stimulated, to a limited extent, the tested C1-bacteria metabolic activity.

3. The very well documented bacterially influenced acidification of the W/J solution supplemented with the ethyl- or the amyl-xanthates suggests the possibility of effective acid-degradation of these substances in the post-industrial environments, rich in various flotation reagents, mainly xanthates.
4. Both the activator containing the carbamate ethyl-derivative and the modifier composed of Cu(II) ions caused complete inactivation of the *A. thiooxidans* C1-metabolism.
5. It is suggested that some unexpected chemical reactions may proceed in the systems tested. They may result from interactions between the culture medium components, the flotation reagents, their decomposition products, and also the products of bacterial metabolism.

ACKNOWLEDGEMENTS

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Pacholewska M., Cwalina B., Steindor K., *Wpływ odczynników flotacyjnych na utleniające siarkę bakterie Acidithiobacillus thiooxidans,* Physicochemical Problems of Mineral Processing, 42 (2008), 37-46 (w jęz. ang)

Wykazano, że różne odczynniki flotacyjne oddziaływały w różny sposób na aktywność metaboliczną utleniających bakterii siarkowych *Acidithiobacillus thiooxidans* (szczep C1 izolowany z wody szlamów Fe-Zn) rosnących w pożywce płynnej Waksmana/Joffe (W/J) z tiosiarczanem jako podstawowym źródłem energii dla wzrostu bakterii. Ksantogeniany: etylowy i amylowy, jak również odczynnik spieniający stymulowały w ograniczonym zakresie aktywność metaboliczną testowanych bakterii C1. Bardzo dobrze udokumentowane, spowodowane przez bakterie zakwaszenie roztworu W/J zawierającego dodatek ksantogenianów: etylowego lub amylowego sugeruje możliwość efektywnej kwaśnej degradacji tych substancji w środowiskach poprzemysłowych bogatych w różne odczynniki flotacyjne, głównie ksantogeniany. Zarówno aktywator zawierający etylową pochodną karbaminianu, jak i modyfikator zawierający jony Cu(II) powodowały zupełną inaktywację metabolizmu *A. thiooxidans* C1. Sugeruje się, że w testowanych systemach mogą przebiegać pewne nieoczekiwane reakcje chemiczne, jako rezultat interakcji między składnikami pożywki, odczynnikami flotacyjnymi, produktami ich rozkładu, a także produktami metabolizmu bakterii.

słowa kluczowe: Acidithiobacillus thiooxidans, aktywność metaboliczna, odczynniki flotacyjne, ksantogeniany, spieniacz, aktywator, jony Cu(II).