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BIOLEACHING OF METALS FROM POLISH BLACK SHALE IN NEUTRAL MEDIUM

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The black shale present in Polish ores (Lubin region) differs from others in mineralogical and chemical properties and in susceptibility to enrichment. The ores are characterized by high content of copper and others metals like silver. Some metals in the shale ore are present as bituminous organo-metallic compounds such as porphyrinus. The presence of these compounds, reduces metals recovery by classical acid bioleaching methods. This study has been undertaken in order to determine possibilities of metals bioleaching from the sandwich compounds occurred in black shale. The bioleaching process was carried out in neutral medium using *Bacillus cereus* and *Bacillus amyloliquefaciens* strains. The progress of this process was followed by analysis of copper, zinc and nickel bioleaching kinetics. The bioleaching experiments with copper suggest different organic forms of this metal in the shale ore. Some of them are highly susceptible to bioleaching by heterotrophic bacteria. Organic compounds of the nickel appeared to be more resistant to bioleaching than copper compounds. Bioleaching of zinc was negligible. Also, the bioleaching process was carried out on a batch scale using "Biomel" reactor.

Key words: bioleaching, black shale ore, Bacillus cereus, Bacillus amyloliquefaciens

INCTRODUCTION

The Lubin deposits of a cupriferous ore consist of three lithological forms: carbonate, sandstone and shale. Shale ores containing a bituminous component are called the black shale. Main components of the black shale are loams, carbonate minerals, organic compounds and detrital minerals [Kucha et al., 1996; Łuszczkiewicz, 2000]. In the black shale some organic matter occurs in a shape of laminas, small inclusions, loamy interlayers and organic remnants. The contents of the

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organic matter fluctuate from 1% to 30% with 6% on average; however, the contents of bitumen range from 1% to 11% [Speczik et al., 1996]. The black shale ores differ from others in mineralogical and chemical properties and in a susceptibility of recovery. They are characterized by high content of copper (5.5 %) and other metals like silver (0.01 %). In the bituminous shale ore the content of metals is from 3 to10 times greater then that in carbonate and sandstone forms [Łuszczkiewicz, 2000]. Therefore this ore can be treated as a natural polymettalic concentrate.

Some metals in the shale ore are present as bituminous organo-mettalic compounds as porphyrinus. The presence of these compounds, reduces the metals recovery by using classical methods of ore enrichment. [Łuszczkiewicz, 2000]

This study has been undertaken to determine the possibilities of bioleaching of metals from sandwich compounds occurring in Lubin (Poland) black shale. The bioleaching process was carried out in neutral medium with *Bacillus* bacteria strains. The progress of this process was followed by analysis of bioleaching kinetics of copper, zinc and nickel recovery.

MATERIALS AND METHODS

MATERIAL

Cupriferous black shale ore was obtained from the Lubin region in Poland. The shale was initially subjected to acid bioleaching, then after extensive washing with distilled water the second bioleaching step was performed under neutral pH condition. Material prepared by this means contained 1.58% Cu, 0.67% Pb, 0.27% Zn and 0.006% Ni.

ISOLATION OF MICROORGANISMS

The bioleaching process was carried out using autochthonous bacteria. *Bacillus* bacteria strains were isolated by our team from the investigated shale samples. Microorganisms were washed out from black shale with physiological solution. The suspended matter was diluted by Lister method from 10^{-1} to 10^{-6}. About 0.1 cm^3 of diluted fluid (10^{-5} and 10^{-6}) was taken from test-tubes and placed on nutrient agar. After 24 hour of aerobic incubation at 30^{o}C, the grown bacterial culture was transferred into flasks with enriched broth.

In order to select *Bacillus* bacterial strains with spores, flasks with bacterial culture were pasteurized [Chmiel, 1998; Kunicki-Goldfinger, 1998]. In the next step of experiment we screened them on the solid medium and transferred onto a thiolignin medium, specific for *Bacillus*, followed by 24 hour aerobic incubation at 30^{o}C. As a result two aerobic, *Bacillus* strains (designed as B_1 and B_2) were obtained and identified. The first step in identification process involved staining using Gramm method [Singleton, 2000]. In all cases Gram (+) batch were observed and then tested on a microanalizer API. The isolated B_1 and B_2 strains were identified as *B. cereus* and designed as B.c.-04, and *B. amyloliquefaciens* designed as B.a.-04, respectively.

This identification was confirmed using Moseley medium. In the case of the B_1 strain the plates were colored red indicating the *Bacillus cereus* strain. Next the cultures were grown on the enriched broth.

BACTERIAL STRAINS CHARACTERISTICS

Bacillus cereus is an aerobic or relatively aerobic, Gram(+) bacterium that can produce resting spores. This bacillary bacterium (3-5 μm in length and 1.0 -1.2 μm in wide) has a flagellum and tends to form chain-like colonies. It grows from 5^o to 50^oC of pH 5.5-8.5 with a water activity of $a_w = 0.95$. Our *B. cereus* strain hydrolyzes casein, starch, gelatin and decomposes glucose and tyrosine. Extracellular products of the bacterium contain hemolysins, lytic (included proteolytic) enzymes, and phospholipase. Some strains of this bacterium under certain conditions produce red or fluorescent yellow-green pigment. [Bergley, 1986]. *B. cereus* strains are frequently found in soil, water and sewage. This bacterium can contaminate food-products and during their growth in food or intestines produce poisonous endotoxins [Prescott et al., 1999; Libudzisz et al., 2000].

Bacillus amyloliqueficiens strain was isolated and identified as a different *Bacillus subtilis* strain by Welker and Campbell in 1967. They found the difference between mole% G+C in DNA of *B. amyloliqueficiens* (43.5-44.9) and DNA of *B.subtilis* (42.0-43.0). The DNA hybridization of these species shows only 15% of homology explaining some different properties of the species. They contain an inducible amylase gene that can produce great amounts of active α-amylase enzyme. *B. amyloliqueficiens* cells are 2-3 μm in length and 0.7-0.8 μm wide. This strain is an aerobic or relatively aerobic, Gram(+) bacteria strain, with resting spores. The temperature of the bacterium growth is in the range 5^o-50^oC, pH = 6-8 [Bergley, 1986].

BIOLEACHING EXPERIMENTS

Bioleaching of metals from black shade was carried out in Erlenmayer flasks on a small laboratory scale. The leaching medium contained 0.75% KNO_3, 0.1% K_2HPO_4 and 0.05% $MgSO_4 * H_2O$. The active bacteria strains found in the culture were *B.c.-04* and *B.a.-04*, in ratio 1:1 and the solid phase to liquid phase ratio was 5:1. Experiments were performed during 28 days in three batches at 10^o, 20^o and 40^oC. The pH value was brought close to pH 7. Three control systems, with thymol as a bacteriostatic substance, were simultaneously prepared.

The progress of the extraction process was monitored by measurements of the concentration of following ions: Cu^{2+} every three-four days, Ni^{2+} in the third and the last day and Zn^{2+} at the end of the experiment. All these measurements were performed using ASA method. An acidity of systems were checked twice a day and adjusted to pH 7 with 10% NaOH.

The bioleaching process was carried out over 24 days in the „Biomel" batch reactor, at 25^oC with a continuous aeration and mixing (300 r.p.m.). Samples of 400

g black shale ore were placed into 3400 cm^3 leaching medium containing Mg^{2+}, SO_4^{2-}, K^+, HPO_4^{2-}, NO_3^- and 600 cm^3 inoculated broth. The active bacteria strains in the culture were *B. cereus* - B.c.-04 and *B. amyloliquefaciens* - B.a.-04, mixed in a ratio of 1:1. The pH value was brought close to pH 7.0 with 10% NaOH. Simultaneously, we prepared control reaction mixtures that contained 400 g black shale and 4000 cm^3 leaching medium with 20 g thymol as a bacteriostatic agent. The concentration of ions was determined every 3-4 days as follows: Cu^{2+} by ASA method, concentration Fe^{2+} and Fe^{3+} by a complexiметric titration, and Ni^{2+} by ASA method when process was finished.

RESULTS AND DISCUSSION

We studied bioleaching of copper, nickel and zinc ions from the black shale during 28 days of process duration. The extension of the leaching process in the studied and control systems at three temperatures is shown in Figure 1, 2 and 3.

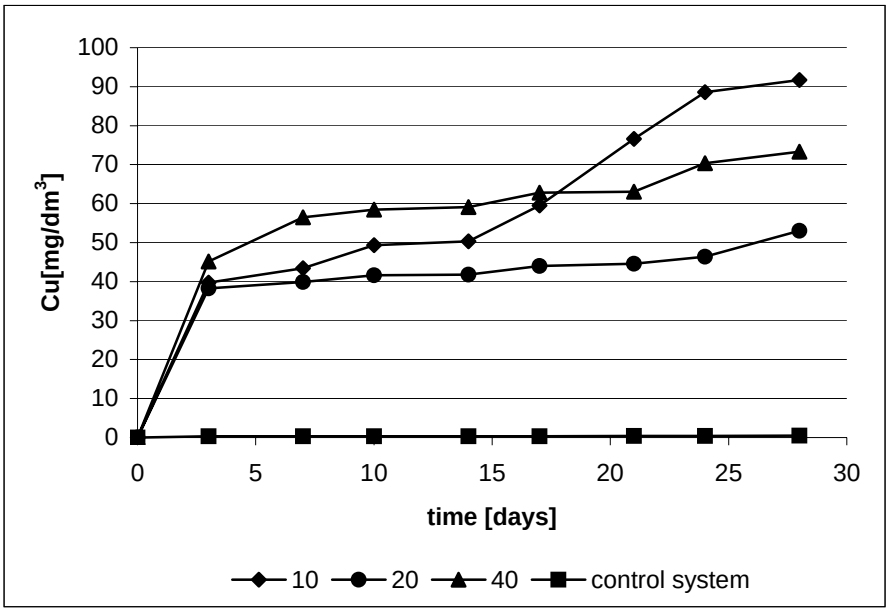

Fig. 1. The effect of temperature on the copper bioleaching during 28 days of process (10^o, 20^o and 40^oC)

In the first three days of leaching, the concentration of copper increased quickly up to 40 mg/dm^3 at the three temperatures. For the next seven days, bioleaching process was not observed (Figure 1) at 10^oC. After 20 days the process started again and the concentration of Cu^{2+} ions increased up to 55 mg/dm^3 (1.7% copper contained in the studying shale). At 20^oC, the leaching of copper stopped after seven days (leaching concentration 59 mg/dm^3). Some leaching was observed again after 20^{th} day of the

process duration (Figure 1). In the last day of studies the concentration of Cu^{2+} ions reached 75 mg/dm^3 (2.4%). The maximum concentration of copper (90 mg/dm^3) was found in the 28th day of the leaching, at 40^{o}C. We were able to extract 2,8% copper contained in the black shale (Figure 1). In all control systems, no leaching of copper was observed that indicating only biological leaching (Figure 1) in the studied samples.

The concentrations of nickel ions were measured only in the third and the last day of the process duration. Figure 2 shows the values obtained for nickel leaching. As can be seen, bioleaching process was not observed in the first three days (at the same time the quantity of leached Cu^{2+} ions was greatest). In the 28th day bioleaching of nickel was highest at 40^{o}C and reached up to level 1.2 mg/dm^3 (10% nickel contained in the studying shale). The levels of leached nickel at 10^o and 20^{o}C were 6% and 9%, respectively (Figure 2).

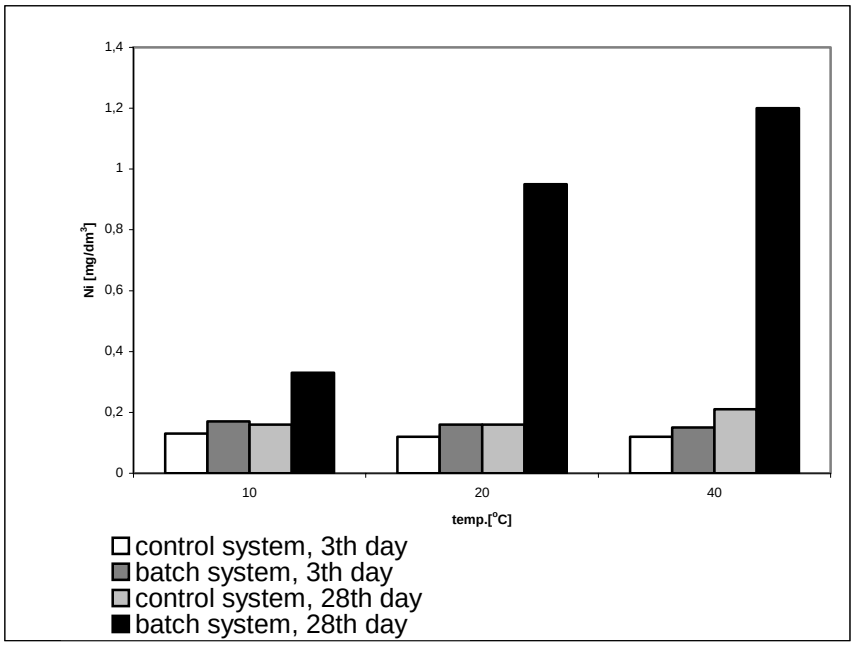

Fig. 2. The effect of temperature on Ni bioleaching during 28 days of process

The concentrations of zinc ions were determined only in the 28th day of process duration (Figure 3). Bioleaching was highest at 40^{o}C but only 0,007% zinc was released.

In order to obtain more information, the bioleaching experiment was carried out on a bench scale. The conditions for bioleaching process were similar with the condition used in laboratory experiments (neutral medium, heterotrophic bacteria *B. cereus* - B.c.-04 and *B. amyloliquefaciens* - B.a.-04). The results are shown in Figure 4.

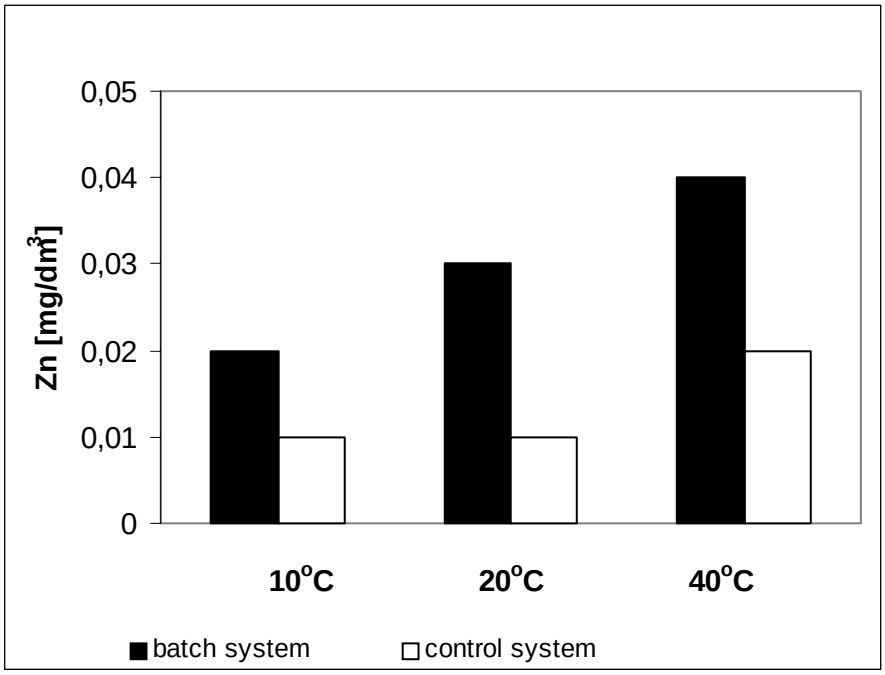

Fig. 3. The bioleaching of Zn during 28 days of process at 10°, 20 ° and 40 °C

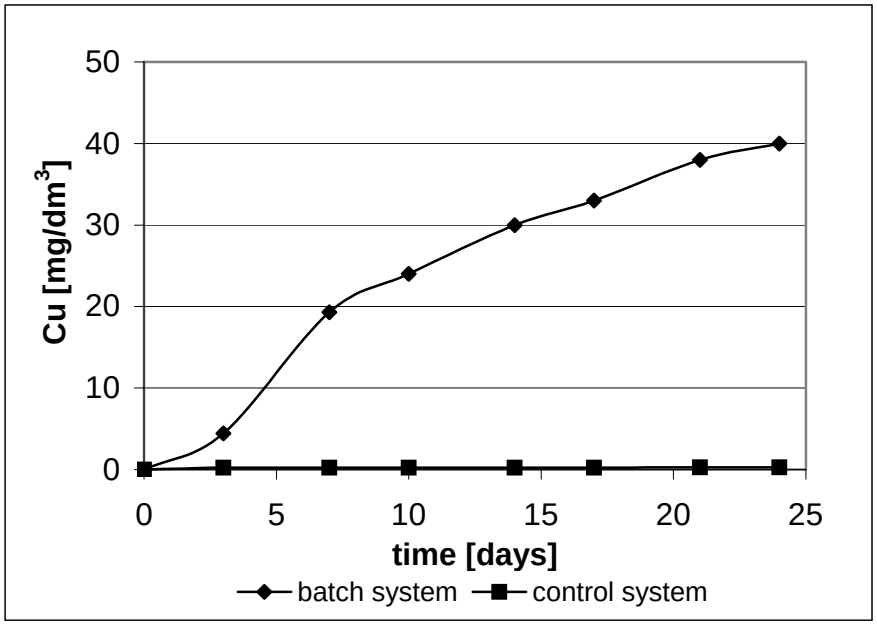

Fig. 4. The bioleaching of copper during 24 days of process

The process of copper leaching started in the 3th day and ran most intensely between the 3rd and 7th day. As shown in Figure 4 the slope of the curve decreases after 7 days indicating that the rate of extraction process decrease after that time. However, there was a significant overall rate of copper production between the 7th and 21st day. In the last three days the reaction was very slow. The first three days represent an adaptation period for microflora. The microflora adapted well to gentle mixing, however it was shocked by intensive mixing in a bioreactor. The intensive mixing was necessary for keeping an uniform ore suspension.

The bioleaching and leaching data are presented in Table 1.

Table 1 Bioleaching and leaching copper and nickel extraction

Process	Recovery , [%]	
	Copper	Nickel
Bioleaching	2.5	9.3
Leaching	0.15	0.22

CONCLUSIONS

The results of our experiments have confirmed predications that copper, nickel and zinc contained in Polish black shale appear in organo-mettalic compounds, because they can be extracted by bioleaching with *B. cereus* and *B. amyloliquefaciens* strains. The bioleaching experiments with copper (Figure 1) suggest different organic forms of this metal in the shale ore. Some of them are highly susceptible to bioleaching by heterotrophic bacteria during the first three days. The others extracted only after 14 days of the process duration at 40°C (Figure 1). We have also found that up to 10% of nickel still remaining in the black shale after acid bioleaching is an organic nickel. The organic compounds of the nickel appeared to be more resistant to bioleaching than the quickly dissolving copper compounds (Figure 2).

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W artykule przedstawiono wyniki izolacji, z polskiego łupka miedzionośnego, autochtonicznych szczepów bakterii heterotroficznych oraz wyniki badań nad bioługowaniem tegoż łupka w środowisku obojętnym, przy współudziale wyizolowanych bakterii. Proces prowadzono w małej skali laboratoryjnej, w trzech różnych temperaturach. Dla porównania, proces bioługowania w większej skali został przeprowadzono w bioreaktorze. Proces prowadzony był w bioreaktorze przez 24 dni w temperaturze 25^0C.