Copper bioleaching from after-flotation waste using microfungi

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Biolúhovanie medi z odpadu po flotácii použitím mikrohúb

The aim of the research presented is an analysis of ways of utilizing microfloral autochthonous organisms from the after-flotation waste of the Gilow stockpile in order to bioleach copper. The alkaline character of the environment disables the use of the traditional processes of acid bioleaching, because of both the economical and environmental aspects. A research of the bioleaching process of the after-flotation waste was conducted using microfungi of the Aspergillus niger *species, which dominate in the autochthonous environment. The metabolism of these microfungi, connected with the production of large amounts of organic acids, allowed to conceptualize their usage in the biohydrometalurgy copper processes.*

After isolating in a pure culture and multiplying the microfungal biomass Aspergillus niger*, the experiments began. Weighed samples of the waste were covered with a selective medium and then inoculated with the microfungal biomass, playing the role of the bioleaching agent. After thirty days of incubation, the end product was chemically analyzed, showing effects of the conducted copper bioleaching process (81,23–87,98 %).*

Key words: copper, bioleaching, microfungi, Aspergillus niger, after-flotation waste

Introduction

In the 1980s, the re-cultivation of the after-flotation waste in the stockpile, located in the so-called LGOM (Legnicko-Glogowski Copper Region), was started. From the beginning of existence of the preparation plant, there was a problem of utilizing this waste. For many years, new ways of its rational utilization have been researched but none of them till now have fulfilled the requested criteria. The first was a concept of utilizing the after-flotation waste in farming. It should to be used as a calcium-magnesium fertilizer, to deacidificate the soils and to utilize unused swampy areas. However, one cannot ignore the presence of heavy metals, which consequently would proliferate and accumulate in the environment. The conducted research shows that, the after-flotation waste should not be used in farming (Lewiński et al., 1996) because of the above-mentioned reasons.

A safer and more effective way would be to utilize the waste in the construction of houses and roads. Experiments conducted between the 1970s and 80s confirmed the usability of the waste as a mineral filling to upgrade bituminous pulps. A site to produce the mineral flour inside the Gilow stockpile was prepared but unfortunately the project was not realized till today. The new concepts regarding the utilization of these wastes deal with their partial usage as a hydraulic filling in the LGOM mines (Kowalska M., 1989).

Because of the relatively high percentage of copper and silver, the Gilow stockpile can be regarded as a secondary deposit site, with the characteristics of a poor placer deposit. The previous metal extraction by the process of acid bioleaching did not provide expected results in this case. In this process, one would have to use very large amounts of sulfuric acid, which in a reaction with the carbonate rock would produce gypsum, insulating the medium in the spot where the chemicals would come into contact. Because of the large consumption of the bioleaching agent and the negative environmental effects, as well as the unparalleled low effectiveness of the process, the usage of classical methods of acid bioleaching would not be viable economically and technologically (Skłodowska A., 2002).

The role of microorganisms in the process of metal extraction from sulfide ores does not currently raise doubts.

Because of geological and mining difficulties, the bacterial bioleaching of ores became a process exploiting poor sulfide and oxygen-sulfide ores, mine dumps, as well as rich ores.

This process boils down to the direct or semi-direct action of micro-organisms of the sulfuric minerals. It is based on the biocatalytic acceleration of the oxygenization processes. As a result, indissoluble metallic sulfides easily turn into dissoluble sulfates and through simple hydrometallurgic and electrochemical methods are transformed into a pure metal.

For many years the mechanism and conditions of bacterial bioleaching have been researched at many institutions in Poland and abroad. Depending on the characteristics of the bioleached material, the process requires checking and occasionally optimizing the bioleaching conditions.

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(Recenzovaná a revidovaná verzia dodaná 29. 9. 2005)

Chemolitotrophic and acidophilic thionic bacteria of the genus *Thiobacillus*, and the specises *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*, turned out to be particularly useful in the process of bacterial bioleaching (Skłodowska A., 2002).

The alkalic character of the Gilowa after-flotation waste makes it impossible to use the well-known process, of metal extraction with the use of thionic bacteria of the genus *Thiobacillus*, which live in acidic environments.

In this work, a particular attention was paid to the possibility of using microfungi *Aspergillus niger* in the process of bioleaching. Their presence and even dominance was shown by the conducted microbiological analysis. A large amount of organic acids, which are a product of the metabolism of the microfungi, indicates potentially effective uses in biohydrometallurgic processes.

Microfungi are heterotrophic organisms. They exist in all ecological niches, e.g. supporting the weathering of rocks as well as the mineralization of materials containg metals. Their development is encouraged by the acidic reaction, the presence of sugars, and the appropriate humidity.

These microorganisms can produce large amounts of organic acids, such as citric, glycolic, oxalic, and other acids which work as chemical solvents and impact the change of the environment's reaction.

Microfungi take part in the circulation of elements in the nature and affect the formation and break-down of minerals commonly present in the environment. The biochemical activity of microfungi is diverse. There are species breaking down proteins, pectins, and cellulose. They all break-down soluble carbohydrates and aliphatic acids. The most interesting ones are the species able to produce large amounts of organic acids, which could be used on an industrial scale in bioleaching processes. Examples of such species are *Penicilium sp., Aspergillus niger, Mucor sp., Yarrovia lypolitica*. It is commonly known that citric acid is produced on an industrial scale by using pure strains of *Aspergillus niger* (Ikram-UL-Hag et al., 2002).

The microfungi, due to their biochemistry and relatively high immunity to hostile factors (pH, temperature, etc.), provide an excellent alternative in the bioleaching of metals, since (for environmental reasons) use the classical chemical methods of acidic or bacterial bioleaching with of *T. ferrooxidans* can not be used.

The mechanism of bioleaching metals through microfungi is different from that by bacteria, which is more complicated. The extraction consists mainly of producing metabolites that are able to bind metals: organic acids, amino acids, and peptides (Skłodowska A. 2000). Currently, microfungi of the *Aspergillus niger* species are used on an industrial scale to bioleach gold from poor deposits and wastes (Chmielewski T., 2002).

Experimental procedures: materials and methods

The feed to the bioleaching process were after-flotation waste samples, which were retrieved from the Gilow stockpile.

They came from three pits which were 1.3 meters deep and separated from one another by 140 meters in a straight line. The samples were collected 30 meters from the dam and marked with the symbols I, II, and III.

During the research it was necessary to recognize the litological composition of the after-flotation waste collected from the places described above (see Tab. 1).

Tab. 1. Litological composition of the after-flotation waste from the Gilow stockpile.

Fraction [mm]	**Yield** [%]	**Sandstone**		**Shale**		**Dolomite**	
		% by quan.	% by weig	% by quan.	% by weig	% by quan	% by weig
+0,280	5,32	64,7	63,9	19,5	19,6	15,8	16,5
0,280- 0,100	87,10	91,9	92,4	1,2	1,2	6,9	6,4
0,100- 0,071	4,73	93,1	2,8	1,3	1,3	5,6	5,9
0,071- 0,045	1,97	97,5	97,4	0,2	0,2	2,3	2,4
- 0,045	0,83	17,7	16,9	-	-	82,3	83,1

Source: Konieczny, Foszcz, 2004r

Another important characteristics of the material, except for the litological composition, is the granulometric composition. This is because of the differences of copper amounts in different granular classes. The granular composition and the average percentage of general copper in particular classes for each of the samples is presented in Tab. 2.

The first step before beginning each biotechnological process is to do a microbiological analysis of the researched environment. Next is to choose the appropriate organisms with characteristics useful in the given process (best if autochthonous) and to create optimal conditions for their functioning. The most frequently used microorganisms have a good adaptability and, consequently, the ability to develop and proliferate even in hostile environmental conditions.

Tab. 2. Granular composition and average percentage of general copper

Number sample	Screen	γ [%]	Cu [%]	Cu general [%]	Contents Ag [g/ Mg]
I	+0,280	5,32	1,04	0,31	64
	0,280- 0,100	87,10	0,28		8
	0,100- 0,071	4,73	0,30		6
	0,071- 0,045	1,97	0,33		6
	- 0,045	0,87	0,40		9
	Σ	100,0	Cu balance	0,32	General: 11
II	+0,280	1,37	1,17	0,30	76
	0,280- 0,100	56,70	0,31		8
	0,100- 0,071	10,80	0,25		6
	0,071- 0,045	10,99	0,18		6
	- 0,04569,28	20,14	0,21		6
	Σ	100,0	Cu balance	0,28	General: 8
III	+0,280	0,83	1,26	0,25	85
	0,280- 0,100	83,44	0,28		8
	0,100- 0,071	7,74	0,18		6
	0,071- 0,045	3,60	0,22		6
	- 0,045	4,38	0,28		8
	Σ	100,0	Cu balance	0,28	General: 8

Source: Konieczny, Foszcz, 2004

One of the dominant micro-organisms existing in all analyzed samples was the microfungus of the *Aspergillus niger* species. Its strains were isolated and brought under the process of proliferating biomass. After obtaining an appropriate amount of microfungal biomass, which is necessary to conduct the research of copper bioleaching of waste, experiments began.

To do this with the after-flotation waste collected from the Gilow stockpile, weighed portions of 10 grams were prepared (in sterile conditions) and replicated three times. Afterwards, they were put in Erlenmeyer flasks and covered with 100 cm^3 of liquid selective medium (Czapek Doxa) to grow microfungi.

Then, into two flasks of each sample, microfungal biomass was inserted, while the third flask was not inoculated—sterile as “0.” The flasks were placed in incubators at temperatures of 28^0C. The cultures were shaken daily to improve the oxidation conditions of the bioleaching.

The process of bioleaching lasted 30 days. After this period of time, the experiments were stopped and the obtained products were prepared for the chemical analysis. To do this, the produced mycelium was discarded and flushed with distilled water in order to remove completely its solid waste. In order to ashen the biomass, it was put in crucibles and placed in a muffle stove.

The obtained ash was weighed on an analytical scale. It was marked and forwarded to the chemical analysis. At the same time, filtration was conducted of the remaining solution in order to separate it from the solid wastes. The solutions were filtrated to scaled flasks and filled with distilled water. The solid material, which remained after the solid filtration, was dried and weighed on an analytical scale.

The remains of the after-flotation waste from the filtrated solutions and the ashed mycelium were forwarded to the chemical analysis to determine how much copper they contained.

Experimental results and discussion

The results of the conducted microbiological analysis show a relatively poor biological life in the researched samples of the after-flotation waste. They showed the presence of both psychrophilic and mezophilic bacteria, microfungi, and very little amounts of denitryficant bacteria. In the analyzed samples, there were no thionic bacteria. This is caused by the alkaline character of the researched material.

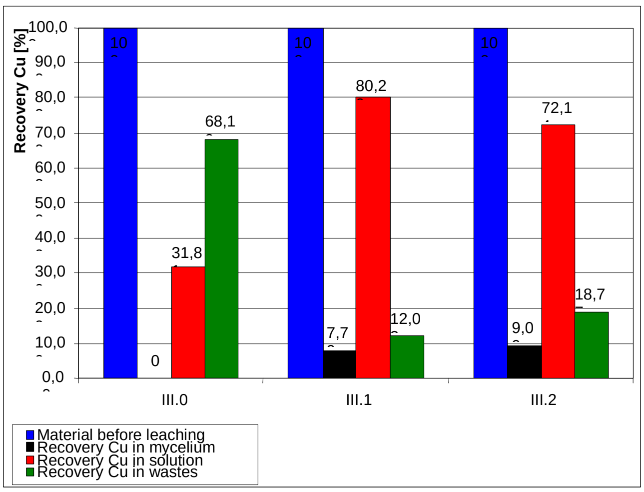

Fig. 1. Graphic presentation of results-sample III

The microfungi were the dominant group of micro-organisms existing in all the samples. With such poor microflora existing in the research environment, the presence of microfungal species, e.g. *Penicillium italicum* and *Aspergillus niger*, is very interesting. These are very valuable species from the point of view of biotechnology.

The chemical analysis of the products—obtained in the experiments of the use of microfungi *Aspergillus niger* in processes of copper bioleaching from the after-flotation waste of the Gilow stockpile—allowed the assessment of the conduct of the process. The effect of bioleaching was assessed by complete obtaining of copper in two products: the solution that was the environment of the bioleaching and the ashed biomass. The third product in this case was the waste, and the copper it contained was a loss of the conducted process (Tab. 3. & 4., Fig. 1).

Tab. 3. Percentage of copper and silver in the wastes after bioleaching

	Symbol sample	**Cu** [%] **(a)**	**Ag** [g/Mg]
1	I/ 0	0,26	9,0
2	II/ 0	0,20	8,0
3	III/ 0	0,20	8,0
4	I/ 1	0,06	8,0
5	I/ 2	0,06	8,0
6	II/ 1	0,04	8,0
7	II/ 2	0,06	9,0
8	III/ 1	0,04	8,0
9	III/ 2	0,06	10,0

Source: E. Kisielowska

Tab. 4. Statement of recoveries from products of bioleaching

	Symbol sample	**Recover Cu from mycelium** ε_B [%]	**Recover Cu from solution** ε_C [%]	**Sum recovers** $\varepsilon_B + \varepsilon_C$ [%]	**Losses Cu in wastes** ε_A [%]
1	I/ 0	-	22,74	22,74	77,26
2	II/ 0	-	32,67	32,67	67,33
3	III/ 0	-	31,81	31,81	68,19
4	I/ 1	8,48	73,78	82,26	17,74
5	I/ 2	8,99	73,89	82,88	17,12
6	II/ 1	7,35	79,54	86,88	13,12
7	II/ 2	10,78	71,69	82,47	17,52
8	III/ 1	7,79	80,20	87,98	12,02
9	III/ 2	9,09	72,14	81,23	18,77

Source: E. Kisielowska

The total obtainment of copper through the process of bioleaching is 81,23 to 87,98 % (Tab. 4., col. 5). The conducted research confirmed the possibility of bioleaching the after-flotation waste by using microfungi of the *Aspergillus niger* species. After the process, the amount of copper in the waste decreased nearly six times.

The obtainment of copper in the solution is 71,69 to 80,20 % (Tab. 4., col. 4.); when accumulated in mycelium it amounts 7,35 to 10,78 % (Tab. 4., col. 3.). A large amount of the copper compunds which were transformed into solutions can be retrieved through serial hydrometallurgic processes (e.g. cementation). The copper absorbed in the microorganisms can be retrieved by putting the mycelium with flotational concentrates and forwarding it to the production of copper stone.

The process of bioleaching that occured with the used microfungi—the metabolites of which were the previously mentioned organic acids, among others—caused the bioleaching solution to lower its pH during the process. In effect, the solution became slightly acidic.

The results of the research conducted for zero trials, without the innoculation with microfungal micro-organisms, indicate a slight decrease in the amount of copper in the waste. The small recovery of copper was obtained through bioleaching the samples with a sterile solution. These effects are probably connected with the presence of soluble forms in the material.

The obtained effects form the basis for conducting a further research of the use of *Aspergillus niger* in the bioleaching processes of the after-flotation waste. This research could be a comparison of bioleaching effectiveness, e.g. in changing temperatures different from optimal temperatures or in changed, slightly acidic, pH. It could form the genesis for conducting bioleaching trials of the after-flotation waste on the industrial scale.

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