STUDY OF VITAL FUNCTIONS, AMOUNT AND OXIDIZING ACTIVITY OF BACTERIA DURING THE BIOLEACHING PROCESS ON HMF-3

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Abstract— Republic of Uzbekistan is on the top-ten list of the gold-mining countries in the world. There are several deposits (Kokpatas, Daugiztau, Zarmitan, Biran, Amantaytau and at all) located in the territory of Uzbekistan, which ores were not involved in production due to the lack of effective technology of their processing despite of the intensive research directed to the development of ore processing.

Index Terms— isolation; sulphur-oxidizing; association of bacteria; flotoconcetrate; of Kokpatas deposit.

1 INTRODUCTION

Nowadays development of novel and environmentally friendly technologies for recycling of mineral raw materials and creation of law-waste or waste-free production is considered to be one of the most important problems in mining countries all over the world. At present time, in worldwide practice pyrometallurgical methods of copper, gold, silver and other precious metals extraction are mainly used. Unfortunately, the disadvantages of pyrometallurgy include formation of dust and gases ejection along with obtaining of products which acquire detoxification and creation of special landfills, which in turn lead to significant environmental pollution by toxic sulphur and ammonia compounds and other toxic elements.

2 PROCEDURE FOR PAPER SUBMISSION

2.1 Purpose of research

Since 2008 Navoi mining combine exploits biohydrometallurgic technology (BIOX) in the hydrometallurgic factory number 3 (HMF-3) in the city of Uchkuduk for processing of refractory arsenic gold ores of Kokpatas deposit.

As a rule, commissioning of a new technology and biotechnology in particular, arises number of problems. Solution of these problems can promote intensification of the process and increase end-to-end non-ferrous metals extraction.

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2.2 Materials and methods

Study of vital functions amount and oxidizing activity of bacteria during the bioleaching process on HMF-3.

For conducting scientific research we performed microbiological assays of selected samples obtained from different sectors of biofactory using inoculation method of limited dilutions. Cultivation of mesophilic iron- and sulfur-oxidizing bacteria we conducted using 9K growth medium (*Acidithiobacillus ferroxidans*), Waksman medium (*Acidithiobacillus thioxidans*) and Baalsrud medium (*Halothiobacillus denitrificans*) at temperature 28-30ºC; for moderate thermophilic iron-oxidizing bacteria we used 9K growth medium with reduced amount of iron (4,8 g/l) with addition of yeast extract (0,02 mg/l) at temperature 43 ºC and 50 ºC. This method allows to detect amount of vital bacteria cells. Incubation period lasts 15 days, titer cells was calculated using McCready's table according to standard methods.

3 TABLE AND SAMPLES

Amount of above mentioned bacteria was determined in following samples, obtained from different sectors of biofactory (HMF):

Table 1

List of samples selected for research in the area of HMF-3

№	№ cipher	Name of the test	pH
1	Reactor 1	Pulp from 31 reactors of the manufactory of biooxidation	pH 1,56
2	Reactor 2	Pulp from 32 reactors of the manufactory of biooxidation	pH 1,35
3	Reactor 3	Pulp from 33 reactors of the manufactory of biooxidation	pH 1,82
4	Reactor 4	Pulp from 34 reactors of the manufactory of biooxidation	pH 1,50
5	Reactor 5	Pulp from 35 reactors of the manufactory of biooxidation	pH 1,45
6	Reactor 6	Pulp from 36 reactors of the manufactory of biooxidation	pH 1,48
7	Reactor 6 biochek	Pulp from 36 reactors of the manufactory of biooxidation	pH 1,48
8	PTD-1	Pulp from unload thickener PTD-1 of the manufactory of biooxidation (lower discharge)	pH 1,24
9	PTD-2	Pulp from unload thickener PTD-2 of the manufactory of biooxidation (lower discharge)	pH 1,86
10	PTD-3	Pulp from unload thickener PTD-3 of the manufactory of biooxidation (lower discharge)	pH 2,56

Research was conducted using following scheme: in purpose of regeneration and activization of geochemistry active microorganism associations stored in museum, medium was inoculated by acidophilic associations and cultivated on thermostatic shaker. After completion of 70% iron oxidation culture was transferred to the fresh medium in ratio 1:9. This procedure was repeated four times. As a result we obtained active acidophilic association. Then sterile medium was inoculated by activated association in the same ratio. Medium was actively bubbled by air during 1 hour. One hour later we selected range of samples for detection of cells amount on different medium, after this procedure bacterial suspension was divided into two equal parts and put in fermenters: into one of them we added concentrate which was acidified to pH 2.0 in ratio 1:4, in the second fermenter we added appropriate medium. Both fermenters were put in thermostat at settled temperature, where cultivation by active medium and pulp bubbling was conducted. Each 24 hours during following cultivation in fermenters we detected amount of vital cells using method of limiting dilutions on appropriate medium; concentration of Fe^{2+} and Fe^{3+} ; pH.

During our scientific research we used standard microbiological methods: amount of cells in 1 ml of sample was determined using method of 10-fold limited dilutions on 9K medium with processing of collected results according to McCready's table; cell morphology in samples of cultural liquid was studied by the method of immersion microscopy; cells culture staining was performed with methylen blue; total amount of inoculation material made up to 5-50%. pH of the cultural liquid was determined using ph-meter "Metter Toledo"; concentration of FeO and Fe_2O_3 in solution was detected using complex metric method with solution of trylone B.

3 RESULTS AND DISCUSSION

For detection of geochemistry active microorganisms of productive association, which oxidizes flotoconcenrate both at the stage of bioleaching and on different sections of washing process we conducted microbial assay of sample № 3-10 using method of

Table 2

The number of viable bacteria in different samples of the bioplast and their oxidative activity in iron

№	pH	Total number of cells	Titer of bacteria on various nutrient media, cells/ml				Concentration of iron, gram /liter		
			A. ferrooxidans	A. thiooxidans	Thiosulfate oxidizing bacteria	Moderate thermophiles	Fe^{3+}	Fe^{2+}	$Fe^{общ}$
1	1,56	$3,5*10^8$	$6,0*10^2$	$2,5*10^3$	-	$6,0*10^7$	29,607	0,973	30,58
2	1,35	$2,5*10^8$	$2,5*10^3$	$2,5*10^3$	-	$6,0*10^7$	29,746	1,39	31,136
3	1,82	$3,7*10^7$	$6,0*10^1$	$6,0*10^3$	-	$2,5*10^6$	14,178	1,112	15,29
4	1,50	$4,3*10^8$	$6,0*10^3$	$1,3*10^3$	-	$1,3*10^8$	16,263	0,834	17,097
5	1,45	$4,2*10^8$	$1,3*10^4$	$2,5*10^4$	-	$6,0*10^7$	19,738	0,695	20,433
6	1,48	$4,9*10^8$	$6,0*10^3$	$6,0*10^4$	-	$2,5*10^9$	22,518	1,39	23,968
7	1,48	$4,9*10^8$	$6,0*10^3$	$2,5*10^3$	-	$2,5*10^9$	23,213	1,39	24,603
8	1,24	$3,3*10^6$	$6,0*10^3$	$6,0*10^5$	$2,5*10^2$	$6,0*10^2$	14,734	-	14,734
9	1,86	$7,5*10^4$	$6,0*10^4$	$6,0*10^4$	$2,5*10^2$	$6,0*10^2$	4,031	-	4,031
10	2,56	$1,2*10^4$	$1,3*10^4$	$6,0*10^3$	$2,5*10^2$	$2,5*10^1$	1,251	-	1,251

limiting dilutions. For cultivation of mesophilic iron- and sulfur-oxidizing bacteria we used 9K growth medium (*Acidithiobacillus ferroxidans*), Waksman medium (*Acidithiobacillus thioxidans*) and Baalsrud medium (*Halothiobacillus denitrificans*) at temperature 28-30ºC and for moderate thermophilic iron-oxidizing bacteria - 9K growth medium with reduced amount of iron (4,8 g/l) with addition of yeast extract (0,02 mg/l) at temperature 50 ºC. Results were presented in table 2.

Conducted microbiological assay showed existence of bacterial cells almost in all studied samples. Worth to mention that data of cells quantification must be considered as semi quantitative analyze. They reflect general tendency of all distribution on the samples and does not provide real data, which is connected in first with time period (24 hours) between samples selection and conducting microbiological assay and second with change of temperature during their delivery to the laboratory. In reality, we can suggest that cells titer in the moment of sample selection was two or three times higher. `

Amount of vital bacteria in different samples of biofactory samples and concentration Fe^{2+} and Fe^{3+} in solution is represented in table 6. Relatively low titer of iron and sulfur oxidizing cells, capable to oxidize iron and sulfur at temperature 28-32ºC (mesophilic) and 43-50 (moderate mesophilic) and also to reduce sulfur compounds (mesophilic) detected in biofactory samples, is connected in first ith time between sample selection and inoculation and temperature fluctuation during the delivery process to Institute of Microbiology. Besides, process of biooxidation on the factory is conducted in regime of continuous cultivation and leaching in condition of hemostat.

It is known that amount of cells in condition of hemostat is directly related to the cultivation regime and velocity of medium passing through the solution in particular.Any changing in condition of living activity and thus periodical cultivation, which takes place during sample selection, leads to sharp reduce in their amount, what subsequently reflects on the results of their analyses. In considerable extent this factors reflect on amount of modern thermophilic cells. Given in table 2 data shows that in spite of above mentioned negative factors in bioreactors, titer of moderate mesophylic bacteria is 2-3 times higher than mesophylic titer. The greatest amount of moderate thermophiles was detected in reactors №4-6. On the contrary, during bacterial keck's washing process, we observed reduce in amount of thermophiles along with simultaneous increase in amount of iron-oxidizing mesoplilic bacteria. Sulfur-oxidizing bacteria are also detected at the stage of concentrate biooxidation and biopulp washing, at the same time development of thiosulfate oxidizing bacteria is observed at the stage of washing process.

In three parallel reactors on the first stage of biooxidation process, concentration of Fe^{3+} came to 29,61; 29,74; 14,18 gram per liter respectively. In reactor №4 with pulp from three reactors, concentration of iron was 16,26 gram per liter, with relatively low pH. Observed increase in iron oxidation in next reactors is quite reasonable and tells about active oxidization of sulfide minerals, what confirmed by simultaneous pH decrease. Presence of insignificant concentration of Fe^{2+} in solution testifies about continues active sulfide oxidation. Decrease in Fe concentration in solution during three washing stages is at the expecting level.

4 CONCLUSION

In bioreactors, titer of moderate mesophiles is two three times higher than amount of mesophiles. The greatest amount of moderate thermophilic cells is detected in reactors №4-6.

On the contrary, during the bacterial pulp washing operation amount of thermyphilic cells decreases simultaneously with increase of iron-oxidzing mesophilic bacteria.

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