ISOLATION OF IRON AND SULPHUR-OXIDIZING ASSOCIATION OF BACTERIA FROM THE FLOTOCONCETRATE OF KOKPATAS DEPOSIT

N.Magbulova
M.Sagdieva
U.Juraeva

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

In recent times attention of metallurgists, geochemists, biotechnologists, microbiologists and other specialists, who involved in different fields of mining industry is drawn to biohydrometallurgy and biogeotechnology. Nowadays, biohydrometallurgy is considered to be one of the most prospective directions of hydrometallurgy. It is characterized not only by economic effectiveness and high environmental friendliness by processing of dredge ore of different kind of dump, but also by its ability to substitute traditional environmentally-unfriendly pyrometallurgical technologies, which used by a number of mining enterprises. In the list of countries using biohydrometallurgic methods of extraction of non-ferrous, precious and rare metals, worth to mention such countries as Republic of Southern Africa, Australia, USA, Canada, Russia, Ghana, Spain, Poland, Bulgaria, Chili, Argentina, China and many others.

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.

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 [1].

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 recovery.

The purpose of research work was isolation of iron and sulphur-oxidizing association of bacteria from the flotoconcetrate of Kokpatas deposit.

2 MATERIALS AND METHODS

For conducting scientific research we performed microbiological assays of selected samples obtained from different sectors of biofactory using inoculation method of limiting dilutions. For cultivation of mesophyll iron- and sulphur-oxidizing bacteria we used 9K growth ambience (Acidithiobacillus ferroxidans), Waksman medium (Acidithiobacillus thioxidans) and Baalsrud medium (Halothiobacillus denitrificans) at temperature 28-30ºC and for moderate thermophilic iron-oxidizing bacteria - 9K growth ambience with reduced amount of iron (4,8 g/l) with addition of yeast extract (0,02 mg/l) at temperature 43 ºC and 50ºC. This method allows to detect amount of vital bacteria cells. Incubation period lasts 15 days, titer cells was calculated using McCredie's table according to standard methods [2-3].

Amount of above mentioned bacteria was determined in following samples, obtained from different sectors of biofactory (HMF-3):

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*

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 ambience 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

Originally, native microflora was isolated from flotation concentrate samples of Kokpatas deposit on 9K ambience at pH 1,4, on shaker with 180 rpm, at temperature 41° C. In this case process of biooxidation of Fe^{2+} to Fe^{3+} lasted very slowly, during 12 days and even longer. Amount of iron-oxidizing bacteria in concentrate was insignificant, iron-oxidizing activity is represented in the table 2.

Table 2

Dynamic of $Fe^{2+}$to Fe^{3+} biooxidation process of the originally isolated native association of iron-oxidizing bacteria obtained from concentrate sample using 9K ambience.

Time of the incubation, (day)	**Fe^{3+}**	**Fe^{2+}**	**Fe^{total}**	**pH**
Initial	1,39	10,147	11,537	1,68
First day	1,529	9,591	11,122	1,67
Fourth day	2,919	7,784	10,703	1,61
Sixth day	3,475	8,34	11,815	1,69
Seventh day	4,587	7,180	11,767	1,76
Ninth day	8,764	2,248	11,012	1,74
Eleventh day	11,08	traces	11,08	1,78
Twelfth day	11,08	-	11,08	1,76

As we can see from the data, represented in the table 2, bioxidation process of concentrate originally isolated association of bacteria lasted 12 days at pH 1,61 - 1,78.

After liquid phase in samples with concentrate first acquired yellow and consequently cognac color and we could observe 100% completed oxidation from $Fe^{2+}$to Fe^{3+} and by microscopy there were detected iron-oxidizing bacteria, we started isolation of active associations among native microflora of iron-oxidizing culture. For this 9K ambience was inoculated with cultural liquid. Incubation was performed at 180 rpm, at temperature 41ºC, pH 2,0-2,05. In progress of experiment we registered the velocity of Fe^{2+} oxidizing process and pH.

Periodical inoculation of cultural liquid on 9K ambience was performed as soon Fe^{2+} completely turned to Fe^{3+} at 41 ºC, pH 2,0-2,05.

Thus, were detected optimal conditions for growth and oxidizing activity of acidophilic association of iron-oxidizing bacteria from the flotoconcetrate of Kokpatas deposit.

Represented process in figure 1 process of biooxidation of Fe^{2+} at different pH. As we can see from the obtained data, represented in the figure in this condition of cultivation can be observed active reproduction of iron-oxidizing bacteria an acceleration of Fe^{2+} oxidation process. At pH 2,0-2,05 process of iron oxidation is completed within 20-22 hours, meanwhile at pH 1,4-1,5 only 1,2 g/l of iron is oxidized, while oxidation completed within 120 hours. Thus, in described conditions of cultivation on 9K ambience we can see observe inverse correlation.

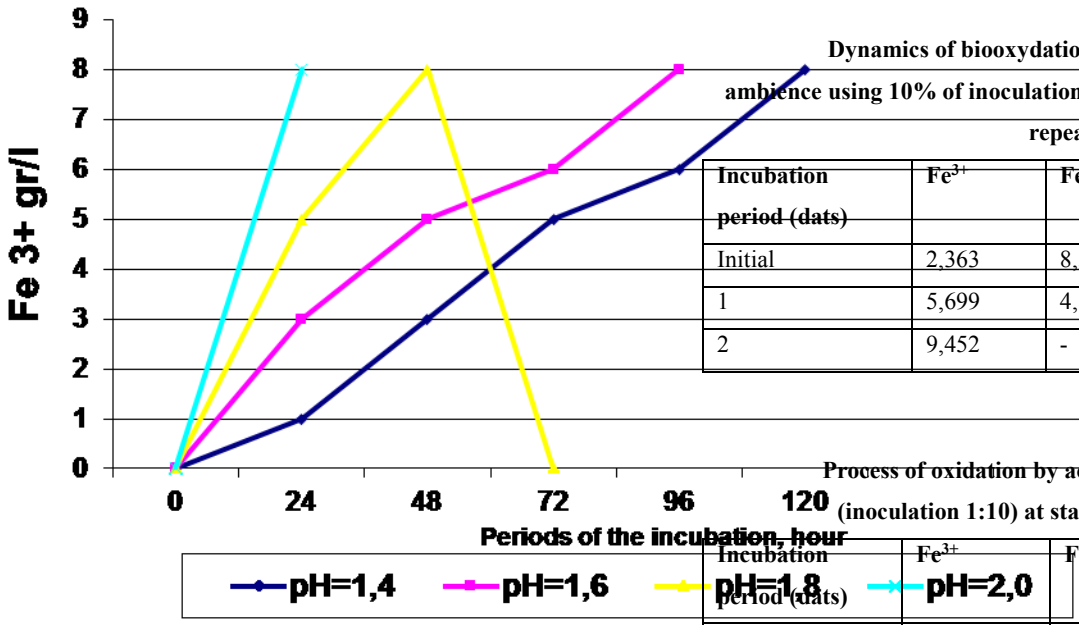

Fig.1 Impact of pH on period of Fe^{2+} biooxidation process, obtained from acidophilic association of iron-oxidizing bacteria concenrate.

Next step of our work consisted of culture activation by inoculation on 9k medium. Inoculation was performed in period of time, when Fe^{2+} could not be detected. In 10-12 repeats of inoculation, cycle of oxidation decreased to 4 days. Given are presented in Table 3.

Table 3

Dynamics of concentrate native microflora biooxydation process (9K ambience) after 10-12 inoculation repeats in ratio 1:1

Incubation period (dats)	Fe^{3+}	Fe^{2+}	Fe^{total}	pH
Initial	3,614	7,089	10,703	1,90
1	4,031	6,506	10,537	1,83
2	4,587	5,811	10,398	1,93
3	10,257	Traces	10,257	1,94
4	10,980	-	10,980	1,98

After obtaining relatively active and homogeny association of bacteria, we started to decrease percent of inoculation material from 50% to 10. In summary, after 10-12 inoculation repeats, period of oxidation of isolated native association in ratio 1:10, shortened to 2 days and then to 20-22 hours. Given are presented in table 4 and 5.

Table 4

Dynamics of biooxydation process of Fe^{2+} to Fe^{3+} in 9K ambience using 10% of inoculation material after 10-12 inoculation repeats.

Incubation period (dats)	Fe^{3+}	Fe^{2+}	Fe^{total}	pH
Initial	2,363	8,506	10,869	1,90
1	5,699	4,865	10,564	1,99
2	9,452	-	9,452	1,98

Table 5

Process of oxidation by active association of Fe^{2+} to Fe^{3+} (inoculation 1:10) at starting titer 10^8cells/ml

Incubation period (dats)	Fe^{3+}	Fe^{2+}	Fe^{total}	pH
Initial	2,448	8,255	10,703	2,05
22 h	10,564	-	10,564	1,87

As we can from represented data, active association of iron-oxidizing bacteria was obtained using method of several inoculation repeats of originally isolated native associative culture on the medium 9K in ratio 1:1 (50% liquid culture+50% 9K ambience) and after 10-12 similar repeats in ratio 1:10 in 9K ambience.

Studies had shown that for relatively short period we manage to obtain an association of very active iron-oxidizing bacteria, which capable to oxidize Fe^{2+} to Fe^{3+} within 20-22 hours and with titer 107-8 cell/ml.

Thus, among isolated microplora of iron-oxidizing bacteria we selected association, characterized by high biotechnological potential.

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